

INCREASING SEED GERMINATION AND QUANTIFYING DROUGHT TOLERANCE IN
Bursera graveolens

By

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To my wife, Emma and my son, Colum

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Abstract of Thesis Presented to the Graduate School
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INCREASING SEED GERMINATION AND QUANTIFYING DROUGHT RESISTANCE IN
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Ecological restoration of abandoned mine lands with native vegetation requires a better understanding of the mechanisms that affect growth and survival of tree seedling in this environment of high levels of sunlight, low levels of soil nutrients, and water stress. The neotropical tree species, *Bursera graveolens*, is adapted to arid, nutrient poor conditions. It is also logged for its aromatic and resinous wood. Its use in restoration projects has been limited by the difficulty of producing planting stock. The objectives of this study were to (1) increase seed germination rates via pretreatments so that nursery managers can produce more planting stock, and (2) quantify physiological responses to drought so that land managers can determine appropriate planting sites and estimate future growth. In this two part study, we determined seed germination under several pretreatments such as sanding, soaking in hot water and acid, and measured drought impacts on biomass allocation, photosynthesis, and plant water relations. The results indicated that none of the treatments had any significant effect on seed germination. The passage of a six to eight month dormancy period equal to the length of the dry season in the tree's habitat was also hypothesized to improve seed germination. It is not. However, it appears that a minimum of 2 months of dry after - ripening, are needed for an acceptable seed germination rate of 25%. Water stressed seedlings (at 2% soil water content) maintained similar

levels of photosynthesis to well-watered seedlings (at 19% soil water content) demonstrating drought tolerance of the species. The information generated will enable natural resource professionals to produce more planting stock and guarantee successful field planting. The next logical step for further research would be to examine the physiological responses to drought of plants growing under tropical field conditions.

CHAPTER 1 INTRODUCTION

Overview

Bursera graveolens (Kunth) Triana and Planch, locally known as Palo Santo, is a small tree of the neotropical dry forests found in Ecuador, Peru and other parts of the Americas. It is a member of the *Burseraceae* family that includes the *Boswellia* and *Commiphora* genera, which produce the raw materials used for the historically important incenses: frankincense and myrrh. *B. graveolens*'s fragrant and resinous wood is highly sought after, potentially putting the tree in danger of extinction. The tree grows on arid and nutrient poor sites. The fragrant wood and the tree's drought hardiness suggest an aptitude for use in projects of ecological restoration in areas that once supported tropical dry forest and commercial potential for plantations dedicated to the production of resin.

Worldwide, tropical dry forests and woodlands are characterized by precipitation between 250 mm and 2000 mm a year (Holdridge, 1978). These forests have a mean temperature of 17°C and are frost free. Evaporation exceeds precipitation (Holdridge, 1978; Murphy & Lugo, 1986). More specifically, Holdridge (1978) defines tropical *dry* forest as forest between 1000mm and 2000mm of annual precipitation and *very dry* tropical forest as having annual precipitation of between 500mm and 1000 mm. He classifies as tropical thorn scrub, which is in essence, degraded tropical dry forest woodland receiving precipitation between 500 and 250mm. Another investigator, Gentry (1995), defines tropical dry forest as areas with an annual precipitation between 600 mm and 1600 mm with a dry season of four to seven months. Areas with less precipitation and longer dry seasons are considered desertic thorn-scrub or tropical desert.

It is the subtropical and tropical dry forests of the world that are quickly disappearing under human pressure. According to Janzen (1988), the tropical dry forests is the tropical

lowland habitat most threatened with destruction; not the tropical rainforest. In Ecuador, less than 1% of the original dry forest currently exists (Sánchez et.al, 2006; Neill & Nunez, 1996).

Murphy and Lugo (1986) state various reasons for this; one being that there just was and is more dry forest to destroy than rainforest. Of the earth's landmass in the tropics, 40 % is dominated by open or closed forest. Of that forested area, 42 % is dry forest, 33% moist forest and only 25% wet or rain forest.

Tropical dry forests the world over have been severely impacted by man, being the center of settlement for thousands of years. They have been preferred by people because they are healthier places to live in when compared to tropical humid forests, easier to clear for agriculture because of shorter trees and the climate is favorable for the raising of livestock. Dry forest soils tend to be more fertile than wet or rainforest soils forest soils due to less leaching of nutrients by rain. There are many species of valuable hardwoods in dry forests such as teak (*Tectona grandis* L. F.), and caoba (*Swietenia macrophylla*, King). In fact, Murphy and Lugo (1986), suggest that we may never know the former true extent of tropical and subtropical dry forests because many savannas, scrub or thorn woodlands are thought to originally be dry forests degraded beyond recognition.

Besides being severely reduced in extent, Ecuadorian dry forests, along with dry forests in coastal Peru, are biologically diverse. They are considered part of the Tumbesian eco-region. The Tumbesian eco-region stretches approximately 113,000 km² along the Pacific coast of South America from northern coastal Ecuador to just north of Lima, Peru (Stattersfield, 1998). The eco-region receives its name from the Peruvian city of Tumbes, which in turn is located on the Pacific coast, close to the frontier of Ecuador and Peru.

These Tumbesian forests support a large quantity of endemic species, both plant and animal. There are 313 woody species in the Tumbesian eco-region. Sixty-six of these are endemic to the region. The reason for this high level of endemism is most likely due to their isolation from the tropical dry forests of the Pacific coast of Central America and the Brazilian Cerrado (Sánchez et al., 2006; Neill & Nunez, 1996).

Ecological Restoration

Worldwide, there has been an increasing interest to restore degraded lands into the type of ecological community that once existed on site prior to degradation. Ideally, that ecological community includes both plants and animals. But owing to the difficulties of reintroducing animal species to areas of their former range, people have tended to focus initial restoration efforts on plant communities. Once a suitable habitat is created, the hope is that the animal community of that habitat or, at least, a somewhat complete suite of native animals will recolonize the now restored site (Hobbs & Harris, 2001; Ruiz-Jaen & Aide, 2005).

The destruction of natural communities such as forests caused by mining is small compared to the destruction caused by clearance of land for agriculture, urban expansion and uncontrolled logging. However, it is still significant. For example, Parrotta and Knowles (1999), state that in Brazil, mining causes the destruction of 2000 to 3000 hectares of forest annually. This figure can be extrapolated to other countries with mining industries. The undesired consequences of forest destruction by mines are many. Landscapes, vegetation, biological diversity and living beings are destroyed as limestone is mined to make cement and rock is ground up to make concrete. What remains behind is unsightly bare hills and could be considered visual pollution. Acid runoff from coal mines lowers water pH, making streams unable to support aquatic life. Cyanide used to extract gold from ores, poison rivers. Soil runoff clogs streams, and covers coral reefs in sediment. Windblown dust cause respiratory illnesses in the

communities that border a mine. These negative impacts have social and economic consequences that can be quantified monetarily. Increased health care costs and the loss of commercial fish stocks are two examples (Clemente et al. 2004; Brady & Weill, 2002).

For those reasons, many countries have enacted laws demanding the minimization of negative impacts during mining, and the restoration or rehabilitation of mining sites afterwards. Mining corporations such as the Brazilian mining company Mineracão Rio Do Norte SA cited in the Parrotta and Knowles (1999) articles or the Swiss- owned HOLCIM Corporation often have a stated policy of mineland restoration.

A specific example would be the Ecuadorian Mining Law of 1991 (Ley Minera 1991 del Ecuador). It states that minelands should be restored or rehabilitated as much as possible, once the extraction of minerals or materials is over. In fact, all mines and quarries are required to possess a tree nursery with this end in mind.

The Ecuadorian city of Guayaquil is located in the Tumbesian eco- region and is home to many limestone quarries. At the present, the most common tree species used for mine restoration in Guayaquil are: neem (*Azadirachta indica*, A.Juss); teak, (*Tectona grandis*, L. f); eucalyptus, (*Eucalyptus camaldulensis*, Dehnh); and the ornamental Benjamin's or Weeping Fig, (*Ficus benjamina*, (L.)). Ornamental trees with showy flowers, such as Flamboyán (*Delonix regia*, Boj. (ex-Hook)-Raj) also known as Acacio Rojo, are popular as well. It also states that restoration projects should use native tree species. All of the above mentioned species are exotic. With the exception of neem, these species do not grow well on mine sites because they require fertile soils and more rainwater than is normally available in Guayaquil. These trees were commonly used for planting because people found them attractive and therefore that is what tree nurseries would produce (Morgan, *personal observation*).

Restoration of degraded tropical lands is difficult. Typical obstacles to restoration are human and livestock pressures, frequent fires, and dominance of exotic grasses. The fact that a site has been previously mined only complicates the problem of restoration. Mining adds soil compaction, the removal of topsoil, and soil profile alteration to the list of obstacles that must be surmounted for successful restoration to occur (Parrotta & Knowles, 1999).

The planting of trees and their successful establishment can accelerate natural forest succession processes by ameliorating unfavorable microclimate and soil conditions, and attract animals that bring seeds of other forest species to the site (Parrotta & Knowles, 1997). Restoration, which could be considered a type of accelerated succession to a desired stage, can fail or be significantly delayed if the wrong species or suite of species is chosen to initiate the process. It is a frequent occurrence that restorationists lack the knowledge of which plant species grow where and why plant species grow in the sites that they do (Parrota & Knowles, 2001). The result of costly failures only discourages further attempts at the restoration of damaged ecosystems (Hobbs & Harris, 2001; Ruiz-Jaen & Aide, 2005).

Justification and Antecedents of Study

As mentioned earlier, *B. graveolens* naturally occurs on arid and nutrient poor areas with thin or nonexistent soils, such as abandoned quarries. However, unassisted colonization and recovery of these sites by trees is slow. By producing planting stock, and most importantly, planting them in the field, ecological restoration can be accelerated.

Regionally, *B. graveolens* is also a tree in danger of extinction due to overexploitation for its valuable aromatic resin. Fortunately, the tree is popular amongst the tree buying public of Ecuador for gardens and patio because it emits an agreeable fragrance. And people will plant trees that they find useful or attractive. These three characteristics suggest an aptitude of the species for initiating forest restoration on abandoned minelands (Morgan, *personal observation*).

Unfortunately, widespread planting has been limited by a lack of available planting stock. Seed germination rates are low and vegetative propagation does not work. That vegetative propagation does not work is surprising, because its congener, *Bursera simaruba*, is easily propagated by cuttings (Navarete-Tindall & Orellana-Nuñez, 2002). Informal experiments performed by the author with both seeds and cuttings performed in the tree nursery of the Cerro Blanco protected forest were not encouraging. Seeds yielded a germination rate of 8% (Morgan, *unpublished data*). Cuttings would sprout leaves and even flower but then die after a year. Evidently the cutting was living off of stored reserves. Dead cuttings, once unearthed, showed no indication of root growth. Even the cuttings upon which the commercially available rooting hormone 1- naphthaleneacetic acid, (more commonly known as “NAA”) were applied, failed to sprout (Morgan, *personal observation*). That vegetative propagation does not work is surprising, because its congener, *Bursera simaruba* (L) Sarg., is easily propagated from cuttings (Navarete-Tindall & Orellana-Nuñez, 2002).

In order to determine *B. graveolens*' successful growth in restoration/reforestation projects, something must first be known about its physiology, and its growth in relation to soil moisture. Once the species drought tolerance has been determined, restoration ecologists will be able to determine which sites will be appropriate for the tree species, and how much supplemental water will be needed in the earliest months or years of forest establishment.

There is almost no scientific literature specifically about *B. graveolens*, so that much information is derived from the personal observations of the primary author who spent eleven years working as a tropical forester in Guayaquil, Ecuador. There is great local support to promote the use of *B. graveolens*, for neotropical mineland restoration projects. *B. graveolens* naturally colonizes arid and nutrient poor sites like abandoned quarries. It permits forest

succession to advance to the next seral stage. Once canopy cover is established, other tree species develop underneath its shade (Morgan, *unpublished data*). It is economically valuable and regionally endangered by unsustainable levels of harvest.

Research Objectives

1. Increase seed germination rates via pretreatments so that nursery managers can produce more planting stock.
2. Quantify physiological responses to drought so that land managers can determine appropriate planting sites and estimate future growth.

Study Site

Although the actual seed germination tests were performed at the University of Florida in Gainesville, the seeds were collected in the Bosque Protector Cerro Blanco, a private forest reserve outside of Guayaquil, Ecuador. The latitude and longitude of Guayaquil are 2.21°S 79.90°W. The Pacific Ocean is nearby.

The city of Guayaquil was founded by the Spanish in 1538 on the site of a pre-existing Native American settlement. Currently, it is Ecuador's largest city and port with close to 2.5 million inhabitants (INEC, 2008). There has been a long history of human occupation in the area, with archeological evidence from the Las Vegas culture dating back to at least 9000 BC.

By extension, the tropical dry forests surrounding Guayaquil have been impacted and degraded by humans for several thousand years. Archeological evidence points to a large pre-Columbian population who practiced agriculture. While a colony of Spain, (16th through early 19th century), the city was an important shipbuilding center for the Royal Spanish Navy with the surrounding forests providing lumber. At present, the rich alluvial soils surrounding Guayaquil support intensive agriculture of rice, sugar cane, and cacao. In recent years, nearby mangroves have been converted to shrimp ponds and the limestone hills have been mined for cement or

earth to fill in mangroves and expand the city. Guayaquil is an active center of environmental degradation. There are an abundance of lands in need of restoration.

The Bosque Protector Cerro Blanco protects 6000 hectares of tropical dry forest in southern terminus of the coastal Chongón - Colonche Mountain range. Elevations range from nearly sea level to 514 meters at its highest point. It is administered by the Fundación Pro-Bosque. Bosque Protector means protected forest in Spanish, and Cerro Blanco means White Mountain.

The climate is tropical with 25°C an average annual temperature, minimum 14°C and maximums of 37°C. Average annual precipitation is 988 mm and is concentrated in the wet season months of December through May. Rain is supplemented by fogs or “garuas” in the summer months of the dry season. Fogs are not an insignificant source of precipitation, although difficult to quantify (Bonifaz & Cornejo, 2004). It must also be mentioned that the Pacific coast of Ecuador is periodically subject to the El Niño Southern Oscillation Event (ENSO) where the amount of rainfall can double, triple, or even quadruple what falls in a normal year (Weather data; INAMHI, BBC).

In Guayaquil, the first real downpour happens in the last week of January or first week of February. The wet season reaches its peak in March, and begins to taper off in April with the last rain in the first week of June. It does not rain again until December. In Ecuador, yearly rainfall decreases as one travels south or west towards the Pacific Ocean and in the city of Guayaquil, it is extremely variable within a very short distance (< 10 km). Yearly rainfall in the northern part of Guayaquil is about 1000 mm and in southern Guayaquil less than 500 mm (Valverde, 1990).

Species Description and Ecology

B. graveolens is a tree that obtains between 8 and 15 m of height at maturity. Diameters of the trunk at breast height range between 30 and 50 cm. It is deciduous with compound leaves.

The bark is smooth and gray, streaked with white, where resin has dripped down from a cut or abrasion. *B. graveolens* has tiny white unisexual flowers; (Valverde, 1990). Many, but not all, taxa of the Burseraceae family are dioecious (Daly, 1993). Opler and Bawa (1978), describe the congener *B. simaruba* (L.) Sarg., as dioecious with a male to female ratio of 1.2 to 1. However, it is not clear whether *B. graveolens* is dioecious or not: there is no obvious visible difference in male or female plants other than the presence or absence of fruit (Morgan, *personal observation*). Recent communications with the taxonomists Xavier Cornejo and Douglas Daly of the New York Botanical Garden, incline towards a belief the species is monoecious.

The fruit is an aril; a small black seed covered by a red pulp (Cornejo, 2004). It is contained in a green capsule 1.2 cm long by 0.6 cm wide, attached to a stalk. The two halves of the capsule fall off when the fruit is ripe. The aril is rich in lipids which makes it attractive to ants, rodents and birds alike (Daly, 1993). Lone individuals, and particularly, small groups of trees emit an agreeable odor similar to anise (Guerrero & López, 1993; Valverde, 1990).

The species is common in dry tropical forests from the Yucatan Peninsula of Mexico, south to Peru, and on the Galapagos Islands of Ecuador (Valverde, 1998). While found at sea level near the Equator, the tree can be found growing at elevations as high as 1500 meters above sea level, particularly in the Andes of Southern Ecuador and northern Peru (Colter & Maas 1999; Sánchez et al., 2006). More specifically, *B. graveolens* grows on rocky, arid, and nutrient poor soils (Guerrero & López 1993; Clark & Clark, 1981).

Around Guayaquil, yearly precipitation decreases as one travels west or south towards the Pacific Ocean. With a decrease in precipitation, tropical *dry* forest becomes *very dry* forest, and then morphs into tropical thorn woodland, tropical desert scrub or tropical desert (Holdridge, 1978). In wetter areas like Cerro Blanco, *B. graveolens* occurs on rocky slopes, ridge tops, and

abandoned quarries. In drier areas, instead of being limited to rocky slopes and other well drained soils, *B. graveolens* now starts to be found on a wide variety of arid soils, and its presence is generalized throughout the landscape (Morgan, *personal observation*).

B. graveolens could be considered an early successional species or long-lived pioneer that gets established in a forest opening and persists in the overstory for many years. It is necessary to make inferences of other studies and field observations. Chazdon (1996) and others treat its congener *Bursera simaruba* (L) Sarg., as an early successional species.. A dendro-chronological study in Peru used a 34 year old *B. graveolens* tree amongst others, to document occurrences of the El Niño- Southern Oscillation from 1954 to 2001 (Rodriguez et. al., 2005). Around the city of Guayaquil, *B. graveolens* trees are found growing on highly disturbed sites such as old quarries and previously clear cut ridge tops. Unpublished data by Morgan reports large *B. graveolens* trees growing on the site of a limestone quarry abandoned some 50 years ago.

Flowering of *B. graveolens* occurs soon after the first gentle rains start around the middle of December. These first rainfall events tend to occur at night or in predawn hours and are very light (< 5mm). Opler et al. (1976), found that light rains in a Venezuelan tropical dry forest triggered flowering in anticipation of the heavier rains that will follow.

Pollination is ambophilous, that is to say, performed by *both* insects and wind. Wind pollination is favored by the absence of rain, low relative humidity, and good air movement. These are common conditions in tropical dry forests (Bullock 1994). Furthermore, these are the predominant climatic conditions at the time of flowering for *B. graveolens*; the brief transitional period between the rainy season and the dry season (Morgan, *personal observation*).

Faergi and van der Pijl (1971), write that in general, the wind is the pollination vector of species with unisexual flowers and those that lack substances that attract birds or other animals. However, Vespidae, (wasps), are attracted to plants with whitish, greenish or yellowish flowers. Flores (2002), reports that the congener *B. simaruba* is pollinated by wasps. This coincides with observations by the primary author of small wasps visiting the scentless white flowers of *B. graveolens*.

Ripe fruit start to appear in the last week of April and continue to be available until the first week of June (Morgan, *personal observation*). The seed capsules dehisce leaving the fruit attached to a stalk and hanging from the branch. The fruits do not all ripen at once but rather in ones and twos (Guerrero and López, 1993; Valverde, 1990).

The fruit either fall to the ground or are eaten by birds on the wing or moving around in the branches. These birds either consume the seeds or disperse them. Those birds that disperse the seeds, either defecate or regurgitate the seeds after digesting the red pulp surrounding them. Unfortunately, the primary author was unable to determine which bird species are consumers and which are dispersers. He only saw unidentifiable birds moving about the branches during periods of fruiting. Maybe fruit eating bats disperse the seeds as well. They are present in the area of Cerro Blanco. Seed dispersal by bats just has not been documented.

Bird dispersal and predation of *B. graveolens* seeds has been documented on the Galapagos Islands for species such as the Galapagos dove, (*Zenaida galapagoensis*), the Galapagos mockingbird (*Nesomimus parvulus*,) (Clark and Clark, 1981) and four species of Darwin's finches (*Geospiza* spp) (Grant and Grant, 1980). Finches normally eat seeds, but the Clarks' (1981), pointed out that Palo Santo has very hard seeds. In the Grants' study (1980), only one species of Darwin finch, the largest one, actually ate the seed. The other finches contented

themselves with the fleshy pulp that covers the seed. However, *B. graveolens* primarily occurs on the mainland South America.

In Mexico, research on the congener, *Bursera fagaroides* (Kunth) Engl., showed that the white eyed vireo (*Vireo griseus*), and the grey catbird (*Dumetella carolinensis*), all ate the fruits of *B. fagaroides*, while the white tipped dove (*Leptotila verreauxii*) may consume the seeds (Ortiz- Pulido & Rico-Gray, 2006). The vireo and the catbird are omnivores, the tyrant-flycatchers (Tyrannidae). The Tyrannidae are the largest bird family on earth, with more than 400 species, and are found only in the Americas. These birds eat insects during the breeding season and while their chicks are growing to provide them with protein, the rest of the year they eat or supplement their diet with fruit. Doves and pigeons eat both seeds and fruit (Gill, 1990).

In the Cerro Blanco protected forest and the Guayaquil area, there are 220 species of birds (Sheets, 2004; Pople et al, 1997; Berg, 1994). Some are exclusively frugivorous, many more are partially frugivorous (or omnivorous) like the tyrant-flycatchers, who have 29 species in Cerro Blanco. Some are granivores, totally or partially, like the finches, grosbeaks and the aptly named seedeaters of the genus *Sporophila*. All or any of these species could be seed dispersers or predators or both.

Obviously the Galapagos mockingbird, Galapagos Dove and the various Galapagos Finch species upon which the Clarks and Grants studied seed dispersal of *B. graveolens* in the Galapagos Islands are not present on the mainland of South America or where the seeds of this experiment were collected. However, there is a congener of the Galapagos dove, the Pacific or West Peruvian Dove (*Zenida meloda*) is present as is the white tipped dove (*Leptotila verreauxii*) cited by Ortiz -Pulido and Rico-Gray, along with 11 other species of dove or

pigeon. . Instead of the white eyed vireo, cited Ortiz-Pulido and Rico-Gray, that consumes fruits of *B. fagaroides*, there is the red eyed vireo (*Vireo olivaceus*).

Once the bird dispersed or fallen seed is on the ground it has to wait approximately six months in the leaf litter until the rainy season comes again in order to germinate. Meanwhile it needs to avoid predation by ants and rodents, (Daly, 1993; Morgan, *personal observation*).

B. graveolens also prepares the way for other species of forest trees to colonize the site. In an unpublished study of forest changes over eighteen years in the Cerro Blanco protected forest, plots with an overstory of *B. graveolens* had saplings of *Simira ecuadorensis* (Standl.) Steyererm. and *Capparidastrum petiolare* (Kunth) Hutch. developing underneath the canopy. These two species are representative of the next phase of forest succession as proven by another set of permanent plots higher up the mountain. These permanent plots are along three ascending gradients of moisture, elevation and successional status (Morgan, *unpublished data*).

Uses and Exploitation

B. graveolens has been used for millennia by people for its resinous wood. The tree's common name, Palo Santo, or literally Holy Tree, refers to its historic use as incense in the liturgy of mass in South American Roman Catholic churches. However, it has been used for millennia by native people for its resinous wood before the arrival of Europeans to the Americas. Its woody material has a strong characteristic spicy sweet and balsamic odor like anise (Sokoup, 1970).

Although its oil is still used in the perfume industry (Yukawa and Iwabuchi, 2004), and the wood's use as incense has enjoyed a revival (Michel Bovo, 2006,*personal communication*), the most common use of *B. graveolens* seems to be is as mosquito repellent. The resin impregnated wood is sold in slivers like the pine heartwood or "fatwood" used as kindling in the United States. Small bundles of *B. graveolens* wood are, for sale in both upscale supermarkets

and traditional city markets of Peru and Ecuador. The tree has also been used in folk medicine, with its resin used as an analgesic, its boiled bark as a diaphoretic and its alcohol exudates used to relieve rheumatism. Another use of the resin is to spread it on the cut umbilical cord of an infant to make it dry up and fall off faster (Valverde, 1998). In coastal Peru, the light weight and soft wood of Palo Santo is used for boxes to pack tomatoes and the fruits of ciruelo (*Spondias purpurea*) (Zevallos, 1999). Newer, less traditional uses are woodcarvings, (Bovo, 2006, *personal communication*) bonsai and ornamental plantings (Morgan, *personal observation*).

Unlike rubber tapping, the extraction of the resin consumes the whole tree. The trees' heartwood is where the fragrant resin accumulates. Mature, senescent trees are cut down or sometimes the stumps of dead trees extracted for this purpose (Yagual, 2005, *personal communication*). The people who perform this activity in Ecuador tend to be economically marginal members of "comunas". A comuna is a legally recognized community of "traditional" or indigenous people in Ecuador.

Preliminary trials in Guayaquil show that Palo Santo also has value in the ecological restoration of arid and rocky sites where other trees have difficulty establishing themselves such as abandoned quarries (Morgan, *unpublished data*). In a small field trial, eight seedlings of *B. graveolens* were planted along with eight seedlings each of two other native drought hardy species, *Tabebuia bilbergii* (Bureau and K. Schum) Standl, and *Geoffroea spinosa* Jacq., on a previously exploited quarry platform. The seedlings were divided into 2 blocks of 3 species and 4 individuals per species. After five years, 85% of the *B. graveolens* saplings were still alive with an average height of 118 cm (Figures 1-1 and Figure 1- 2).

All the *T. bilbergii* seedlings were dead. The *G. spinosa* seedlings had a survival rate of 50% and a mean height of 72 cm. This is somewhat surprising, since *G. spinosa* is a legume

adapted to arid conditions. The primary investigator hypothesized that survival of *G. spinosa* would be higher than it actually was.

Conclusion

B. graveolens has the potential to restore abandoned mine spoil sites and facilitate forest succession in areas that were originally tropical dry forests. Increased production of seedlings in tree nurseries could not only be used in ecological restoration projects, but also in commercial plantations to produce oil and resin for the perfume industry. These same plantations could be used to provide work and saleable products to impoverished communities in the regions where *B. graveolens* grows. The traditional beliefs and customs that surround *B. graveolens* can continue because the species will not be extinct.

Table 1-1. Mean (standard error) initial and final height of each species. Also, percent survival after 5 years. Initial heights recorded in 2000. Final height recorded in 2005 except for *T. bilbergii* which w recorded in 2001; by 2005 all the *T. bilbergii* seedlings had died.

Species	Family	Initial Height (cm)	Final Height (cm)	% Survival 2005
<i>Bursera graveolens</i>	Burseraceae	23.6 (4.1)	117.6 (14.6)	88%
<i>Geoffroea spinosa</i>	Fabaceae	24.4 (9.2)	71.8 (26.5)	50%
<i>Tabebuia bilbergii</i>	Bignonaceae	19.6 (1.6)	11.6 (3)	0 %

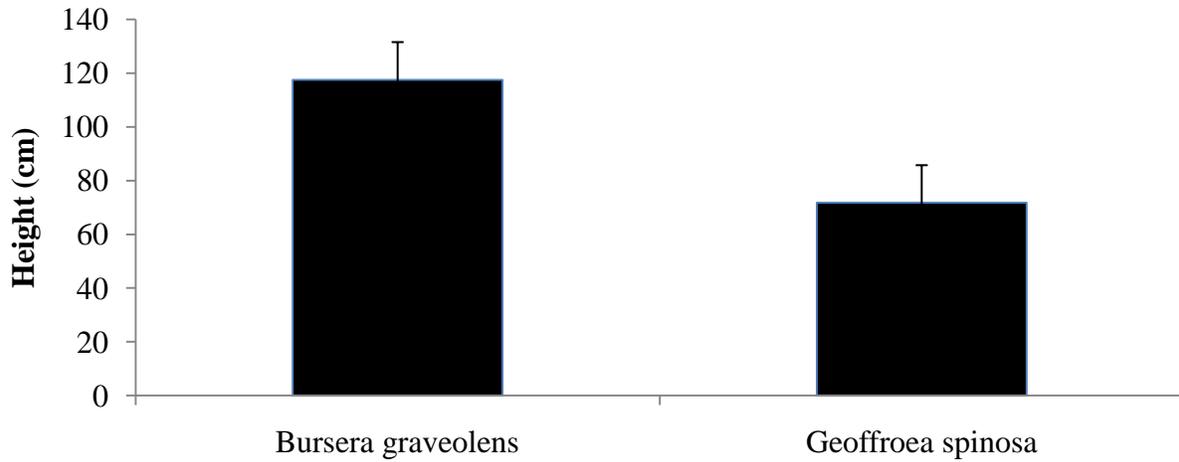


Figure 1-1. Comparison of height growth of *Bursera graveolens* and *Geoffroea spinosa* after 5 years. ($p=.06$). Error bars measure SE

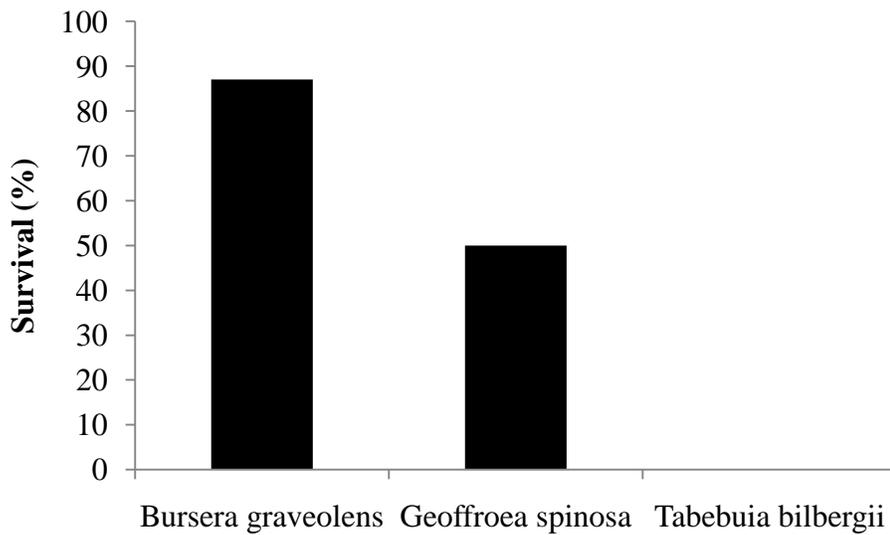


Figure 1-2. Comparison of survival rates of 3 species after 5 years in a small field trial. No error bars because sample size too small.

CHAPTER 2
INCREASING SEED GERMINATION OF *Bursera graveolens*, A PROMISING TREE FOR
THE RESTORATION OF TROPICAL DRY FORESTS

Introduction

Bursera graveolens is a deciduous tree species native to the tropical dry forests of Ecuador and Peru. It grows on arid and nutrient poor sites (Guerrero & López, 1993; Valverde, 1990). The wood was traditionally burnt as incense and mosquito repellent because it possesses aromatic resins (Valverde, 1990; Soudkoup, 1970). In recent years, these resins and oils are being extracted from the wood by the perfume industry (Yukawa & Iwabuchi, 2004).

The tree is a relatively fast growing species that colonizes rocky outcrops. Not only does the tree produce a commercially valuable product, it shows potential for use in the ecological restoration of tropical lowlands. One obstacle to the wider adoption of this species for ecological restoration projects is its low germination rate, and by extension, availability as planting stock (Morgan, *personal observation*). The purpose of this study is to discover the most effective way to increase the germination rates of seeds via treatments before planting.

In general, plants reproduce themselves by exchanging genetic material in the form of pollen with other plants of the same species. From the successful exchange of genetic material, seeds are formed. The seeds, once in the soil, start to germinate when there is sufficient light, air and moisture. The seed coat breaks open, and the seedling puts out a root to anchor itself into the soil and absorb nutrients. Then, two primitive leaves or cotyledons emerge. The cotyledons provide sustenance through either stored reserves or photosynthetic material. After the expansion of the cotyledon and establishment of the root, new photosynthetically capable leaves develop to harness the power of the sun and atmospheric CO₂ in order to grow. The plant is on its long, perilous journey to adulthood and reproduction. Few plants survive the journey.

Seeds, once fully formed, need to get from the branch to the soil. There are several ways of doing so. The most obvious way is to fall off the plant onto the ground below. However, this is not necessarily the best option for the seed. Many seeds and seedlings will not germinate under the shade of its parent(s). The shady microsite underneath the parent might favor the development of pathogens that prey on seeds and seedlings. Or a concentration of succulent seedlings will attract predators; in this case, herbivores.

Therefore, many plants have evolved or devised ways for their offspring to be transported away from the parent and to a (hopefully) suitable site for germination, establishment, growth, and future reproduction. Many trees produce light, windborne seeds that are carried away in the wind some distance. Two examples of trees with wind dispersed seeds are *Cordia alliodora* (Ruiz and Pav.) Oken, and *Cavanillesia platanifolia* (H.and.B.) H.B.K. . Dispersal distances can vary from kilometers to meters. Red Mangroves (*Rhizophora mangle* L.) and Coconuts (*Cocos nucifera* L.) float on water and use the ocean waves and currents to carry the seeds to a suitable site. Other species use animals to transport the seeds. Some seeds are sticky like beggartick (*Bidens frondosa* L.) and attach themselves to animals' fur to carry them away. Other plants use edible fruits to attract dispersing animals. The seeds of Algarrobo (*Prosopis juliflora*) are contained within a sweet pod that ruminant animals, such as cows eat. The seeds are either spit out while the animal chews its cud or defecated later. Walnuts (*Juglans spp*) produce big nuts that are collected and cached by squirrels (*Sciurus spp*) to eat later some distance away from the parent tree. Sometimes the animal does not return for its seeds and a seedling sprouts from the forgotten cache. Birds also disperse many seeds. They are attracted to the ripe fruits and they either defecate or regurgitate the seeds. Two examples of fruit eating and dispersing birds are jays (various genera in the *Corvidae* family) and pigeons (*Columbidae* family). It must also be

mentioned, that often times seed dispersers can be seed predators as well. Squirrels and pacas (*Agouti paca*) eat the seeds they store. It is only when they forget or are unable to return to their seed caches that the seeds germinate.

Some fresh seeds do not germinate when planted in conditions with appropriate light, moisture, temperature and aeration. These seeds are said to be dormant. In order to germinate, it is necessary to break that dormancy.

There are two types of dormancy: chemical and seed coat dormancy. The whole point of chemical dormancy is to avoid having the seed germinate during a brief window of favorable conditions only for the tender seedling to get killed when conditions revert to being freezing or rainless again. The classic example of chemical dormancy is that of the acorns from some northern Oak (*Quercus* sp) species. These seeds pass the winter months under the snow and leaf litter where it is cool and damp before they germinate in the spring. In fact, these seeds will not germinate in a nursery if planted immediately or soon after collection. To get the acorns to germinate, they need to be stored some weeks in a refrigerator in a plastic bag full of wet leaves. Therefore, in this case, chemical dormancy prevents an acorn from sprouting in an unexpected winter warm spell and the seedling killed when conditions revert to freezing again (Smith et al., 2002).

In the case of *B. graveolens*, we would expect that if there is a seed dormancy period, the best seed germination would happen some six months after fruiting and seed fall, at the start of the rainy season. Peak fruiting occurs in May and the rainy season starts up again in December in coastal Ecuador. However, most tropical seeds can germinate readily after seed fall provided there is sufficient moisture available (Smith et al., 2002).

Many seeds have hard seed coats that need to be broken to allow the entrance of water, so that the seed embryo can imbibe water and start metabolizing. This is physical or seed coat dormancy. Many of these seeds are animal dispersed. The seed coat protects the seed embryo, while the overlying fruit is being consumed, allowing for later dispersal. We know that *B. graveolens* produces a flesh covered seed that is defecated by frugivorous birds. This implies that the seed coat that needs to be broken before the seeds can germinate (Smith, 2002). The seed coat is hard because if it was not, the embryo would not be able to survive passage through the gastro-intestinal tract. Not only do birds have stomach acid, they have rough gizzards, sometimes filled with stony grit to help them grind up and digest their food (Gill, 1990).

Various techniques have been mentioned in nursery literature to scarify seeds. One way is to use sandpaper on a seed until it loses its shine, because the oily lipids that seal the seed to water have been abraded away. Another is to crack the seed with a hammer (Vosso, 2002). Maybe this is to imitate the grinding action of the gizzard. Acid baths and hot water soaks have been used to imitate stomach acids (Vosso, 2002). They also allow one to scarify or pretreat many seeds at once. The problem with this method is that the seed embryo can be damaged or killed by soaking too long (i.e. be cooked). It is also potentially dangerous, because hot water and acid can spill and burn nursery staff. A safer method is to soak seeds in cool water for some hours or days so that chemicals that inhibit germination leach out and the seeds imbibe water. One should change the water every day to get rid of leachate or pathogens (Vosso, 2002). The draw back with this method is that seeds can also soak too long and start to rot. For example, Cascol (*Caesalpinia paipai*) Ruiz Lopez and Pavon, is a hard-coated seed from a tropical dry forest tree. The seed is found in woody pods eaten by ruminants such as cattle or deer. One would think that soaking overnight would be an appropriate pretreatment. However, soaking for

more than four hours leads to rotting seeds (Morgan, *unpublished data*). Some more unconventional pretreatments have been feeding seeds to livestock, or even birds, and collecting the defecated or regurgitated seeds, setting fire to the seeds to burn off a thick pericarp, allowing ants to eat the pericarp and even treating the seeds with fungal spores (CATIE, 1993).

I would now like to discuss the findings of other researchers working with congeners of *B. graveolens* and other bird dispersed tree species. *Bursera simaruba* does not need scarification. Seed germination is between 80- 100% (Navarette –Tindall, 1990). Not only is it dispersed by birds, but also by monkeys.

Murray et al. (1994) experimented with another bird dispersed tree species, (*Witheingia*) and the black faced solitaire (*Myadastestes melanops*) which is a type of thrush. In this experiment, Murray and his associates wanted to find out if the fruit of *Witheingia* had a laxative effect, while increasing seed germination. They found that the longer the seed was in a bird's digestive tract, the less likely it was to germinate. However, sixty-two percent of the seeds passed through a bird's stomach germinated, as opposed to mature seeds just picked off the tree (51%). In this case, bird treated seeds germinated a little bit better than untreated seeds.

Perhaps in some cases, frugivory is more important as a means of seed dispersal than as pregerminative treatment. This appears to be the case with *Bursera fagaroides*. Ortiz -Pulido and Rico-Gray (2006), mention that 17% of *B. fagaroides* seeds germinated if eaten and defecated by *Dumetella carolinensis* and 0% germinate if eaten and defecated by *Vireo griseus*. This was actually lower than the germination rate observed for seeds without any treatment (20%).

The objectives of this study were to: 1) determine the most effective treatment to increase seed germination and 2) To determine if there is a period of seed dormancy that needs to pass before germination can occur. We hypothesize that a pretreatment that mimics the passage of a

seed through the digestive tract of a bird will increase seed germination, and that a time period equal to the length of the coastal Ecuadorian dry season must pass before the seeds germinate

Materials and Methods

The seed source for the experiment is a small stand of 30 trees on a rocky slope in the Cerro Blanco Protected Forest. That slope was quarried approximately 40 years ago for limestone. The stand is at an elevation of approximately 80 to 100 m above sea level.

Seeds were collected by the nursery staff of Fundación Pro-Bosque in the spring of 2006. Seed collection began when the fruits started to ripen in the end of April 2006 and continued until June. The fruits were collected by hand on the lower branches and those on higher branches were collected with a pole mounted pruning shear. The fruits do not ripen all at once. Strictly speaking, a fruit's seed capsule opens and falls to the ground, presenting the seed and its red aril to dispersers in ones and twos. It was necessary to return daily or every other day to collect seeds as they ripened. It was assumed that if a fruit was ripe, so were its immediate neighbors and those were collected as well. The collected fruits were put on a table in a shed to dry and the seeds removed from the fruit capsules as they opened.

Seeds were washed in a 10% household bleach solution, (3-6% sodium hypochlorite, NaOCl), to remove the pulp that surrounds the seeds, and then air dried. The bleach serves to disinfect the seeds of pathogens. Seeds were stored in sealed jars in a dry and dark place until permits could be obtained for their transport to the United States. Permitting took approximately two months.

The germination trials were performed in a glass house at the University of Florida in Gainesville. The glass house is cooled by a system of cardboard baffles on one wall. They are kept wet, and the evaporation of water cools the glass house. In the winter, there is no heating provided but for the day's heat trapped under the glass roof of the greenhouse. There are

temperature sensors that will alert the greenhouse manager if temperatures fall below 0°C, and will report the current temperature if one makes a phone call to the greenhouse. There is, however, no permanent record of daily temperatures. The advantage of using a green house and planting pots for the studies are that soil differences can be minimized and the amount of water applied to both seeds and plants will be uniform. In a field study, soil characteristics can vary in less than a meter, rain fall events do not deliver the same amount of rain every time, and the amount of water that can be used by the plant varies with microsite and soil.

Once at the University of Florida, seeds were divided into three batches and subjected to different pregerminative treatments. Ineffective treatments were not repeated for later tests. In the first trial, six month old seed were subjected to four treatments including: 1) a control, 2) physical scarification with sand paper, 3) soaking in 95% pure sulfuric acid (H_2SO_4) for a period of four minutes and 4) soaking for 24 hours in water first heated to 50°C. The seeds were placed in the hot water and allowed to soak as the water cooled. The control treatment consisted of untreated seeds (no soaking in water or acid, or scarification, just dry seeds) planted in the peat or in later tests, Petri dishes filled with vermiculite and sand.

The first trial started on March 16th, 2007 and continued until May 7th, 2007. The trial was performed in a growth chamber to avoid exposure of seedlings to freezing winter temperatures. The chamber was illuminated for twelve hours daily and the temperature maintained at 25°C. Commercially available trays of pressed peat pellets bought at a garden center were used as the germination substrate. Each tray was divided into 36 compartments in which one seedling could be grown. Thirty six seeds in one tray comprised a single replication and each treatment consisted of three replications. The second and third trials were performed in a greenhouse. The substrate used was a 1:1 mixture of sand and vermiculite in Petri dishes. As in the first test, the

substrates were kept moist at all times. Temperatures in green house ranged between 25°C and 40°C.

The second trial started on June 8th, 2007, and ended on August 11th of 2007. It consisted of subjecting year old seeds to immersion in either four treatments of sulfuric acid bath and three of hot water and a control. The acid and concentration used was the same as in the first test, 95% pure H₂SO₄. The acid treatments were soaking for one, two, three and four minutes. Seeds treated with hot water were soaked for 24 hours with initial water temperatures of 50°C, 60°C or 70°C. Thereafter the water was allowed to cool to room temperature. Each treatment had four replications with 15 seeds in each replication.

The third trial started on August 17th 2007 and continued until October 10th, 2007. It consisted of subjecting relatively fresh seeds collected in 2007 to a range of hot water temperatures: 50°C, 60°C, 70°C, 80°C, 90°C, and 100°C. After immersion the water was allowed to cool down and the seeds soak for 24 hours. Once again there was a control treatment. Each treatment had four replications (Petri dishes) with 15 seeds in each. Therefore each treatment had 60 seeds. The only exception to this was the 100°C treatment. There were not sufficient seeds for a complete treatment and it was believed that boiling 100°C water would only destroy the seed embryo. It had one replication with 11 seeds.

Due to what were considered unexpected results with the third trial, a fourth trial was performed from November 1st of 2008 until February 1st, 2009 with increased statistical rigor. A new batch of seeds was received from Ecuador. Eight replications were used instead of four and care was taken to avoid pseudo-replications. Treatments consisted of a control, a range of hot water temperatures from 50°C to 100°C, and acid treatments from 1 to 5 minutes.

Statistical Analysis

Each Petri dish was considered an experimental unit within the framework of a randomized complete block design. Treatments were considered as blocks. Data was checked for to see if it had a normal distribution. If the distribution was normal, the number of seeds germinated in a petri dish was converted into a proportion and then transformed by taking the square root of the proportion and then multiplying the resulting number by the arcsine (Pereira de Souza & Válio, 2001; Chen & Maun, 1998). Only then could an Analysis of Variance be performed upon the data.

If the data did not have a normal distribution, as in the case of trials 3 and 4, the non parametric Kruskal–Wallis test was used to test the treatments for significance. With this test, it is not necessary to transform the proportion of seeds germinated in each petri dish. Finally, treatment means were compared using Dunnett’s Post Hoc test for treatments and controls, and Tukeys Pos Hoc for comparing means within treatments (Ott & Longknecker, 2004). The statistical program JMP-IN 5.1 for Windows (SAS Institute, Cary, NC, USA) was used to perform the analyses.

Results

The results of the four experiments along with their descriptive statistics are summarized in Tables 2- 1 and 2-2. There are no significant differences in germination between the control treatment seeds of the four trials. The two months old, five month old, six months old, and one year old batches of seeds all share similar percentages of germination: 23.3, 18, 20, and 24.1 %. Neither seed age nor seed quality of different lots plays a factor in the germination of these seeds. Therefore, dormancy does not play a role in the inhibition of germination of *B. graveolens* seeds (Figure 2-1).

In the first test, physical scarification gave the best result with 34% topping the hot water treatment at 50°C, which had a 28% germination rate. Four minutes of the acid treatment gave almost the same result as the control (23% versus 24%). Although, physical scarification gave the best results, it was considered too labor intensive for practical application in small nurseries, nor was the difference between the physical scarification treatment and the 50°C hot water treatment statistically significant (Figure 2-2).

The second trial compared different acid treatments with each other and with different temperatures of hot water. There appeared to be no significant differences amongst the different acid treatments and the control. The hot water at 70°C treatment gave the best results with an average germination of 53%. The test results suggest that the hot water treatments were effective at breaking the seed coat and promoting germination (Figure 2-3).

In the third trial, we wanted to determine what water temperature would be optimum to promote seed germination. In the second trial, the highest temperature, 70°C gave the best results. Evidently, temperatures higher than 70°C damage the seeds. Unfortunately in the third trial, for unknown reasons, the seeds in the control treatment had the best germination rate at 23%. Amongst the hot water treatments, 70°C worked the best with 20% average germination. Germination declined at 80°C and ceased at 90°C. The seeds treated with 60°C had a germination of 13%, whereas both the 50° and 80°C treatments were tied at approximately 12%. No seeds germinated in the 90° C treatment (Figure 2-4). Importantly, if the 90°C data is removed from statistical analysis there is no difference between treatments.

Due to the contradictory results of the third trial, another batch of seeds were obtained and planted with more replications, (eight instead of four), and efforts made to avoid pseudo-

replications. Once again the control seeds have the highest overall germination. Both acid treated and hot water soaked seeds had lower average germination rates than the controls.

Discussion

Before we discuss the results of the four experiments, we would like to mention the informal experiments on the seeds carried out in Ecuador. As mentioned earlier, the primary author managed the tree nursery of Fundación Pro-Bosque. He would carry out informal experiments in the nursery. He first planted *B. graveolens* in 1996. Every year he would plant some batches of seeds in some earth filled bins and see what would come up. He would record the number of seeds planted and note the percentage of germination. Some years he varied pregerminative treatments. All seeds were planted soon after collection to avoid problems with seed degradation due to heat and atmospheric humidity, seed predation by larva within the seed or by mice within the store room. Germination was considered unacceptably low from a nursery manager's view point, at 8%. There could be various reasons for what a nursery manager would consider an unacceptable rate of germination; seed predation by fungus or insects in the soil perhaps. Maybe the seeds needed to undergo a period of dormancy.

We were not expecting to find the control do better than all the other treatments. The control of the third test was comparable to the control treatment of the fourth, second and first test with germination rates hovering around 22%. This proved two things. One, there is no physiological dormancy. Once the seed coat is broken, and the seed embryos absorb water, the seeds can germinate. Secondly, the third batch of seeds was not defective in anyway; for example, by being collected before they were mature. If that was the case, we would expect a much lower rate of germination of the controls such as 8 % instead of 20%.

The results of the four experiments show the importance of having more replications of treatments. There are plans to repeat the experiment again under more statistically rigorous

conditions. It also appears that storing the seeds for a minimum of two months and allowing them to dry increases seed germination from 8% in fresh seeds planted soon after collection to about 22% for seeds stored for 2 months, 5 months, 6 months, and 12 months, respectively. This needs to be statistically confirmed in a later experiment performed with fresh seed.

Conclusions

Physical scarification of the seed coat with sand paper yielded the best results with seed germination. Unfortunately it is too laborious a method to use on a large scale until a more efficient method is devised. The control treatment gave the next best results. Until a more efficient method is found to crack the seed coat of many seeds at the same time, planting untreated seeds a 2 month period of dry after-ripening is the most effective way to germinate seeds.

Both acid and hot water soaks usually performed as well as the control or worse. Hot water soaks at 90°C and above are fatal to the seeds. The significantly increased germination of seeds during one trial with the 70°C hot water soak appears to have been a statistical fluke that was not repeated in two further tests. It could be that birds serve more as seed dispersal agents than agents of seed scarification and increased germination.

Table 2-1. Percent germination of seeds for each trial and treatment.

TRIAL	TREATMENTS	MEAN	MIN	MAX	SE	# SEEDS	REPLICATES	Total seeds
1	Control 6 mo	24.07	19.44	30.56	2.72	36	3	118
1	Acid 4 min	23.15	22.22	25.00	0.76	36	3	118
1	Sand Paper	34.26	33.33	36.11	0.76	36	3	118
1	Hot Water 50 C	28.70	25.00	30.56	1.51	36	3	118
2	Control 2 mo	23.25	13.00	33.00	4.33	15	4	60
2	Acid 1 min	23.33	12.50	33.33	4.45	15	4	60
2	Acid 3 min	11.67	6.67	18.75	2.94	15	4	60
2	Acid 2 min	20.00	6.25	31.25	5.63	15	4	60
2	Acid 4 min	21.67	12.50	33.33	4.42	15	4	60
2	Hot water 50 C	21.67	0.00	37.50	7.81	15	4	60
2	Hot water 60 C	36.67	20.00	56.25	8.07	15	4	60
2	Hot water 70 C	53.33	33.33	75.00	8.85	15	4	60
2	Control 12 mo	20.00	12.50	31.25	4.43	15	4	60
3	Hot Water 50 C	11.75	7.00	20.00	3.09	15	4	60
3	Hot Water 60 C	13.25	7.00	20.00	2.66	15	4	60
3	Hot Water 70 C	19.75	13.00	33.00	4.71	15	4	60
3	Hot Water 80 C	11.50	0.00	20.00	4.17	15	4	60
3	Hot Water 90 C	0.00	0.00	0.00	0.00	15	4	60
3	Hot water 100 C	0.00	0.00	0.00	0.00	11	1	11
4	Control 5 mo	18	20.00	6.67	3.5	15	8	120
4	Acid 1 min	5	13.33	0.00	2.7	15	8	120
4	Acid 2 min	14	26.67	0.00	3.4	15	9	135
4	Acid 3 min	6.6	20.00	0.00	2.1	15	8	120
4	Acid 4 min	10	20.00	6.67	1.7	15	8	120
4	Acid 5 min	10	13.33	6.67	3.3	15	2	30
4	Hot Water 50 C	17.5	33.33	6.67	3.7	15	8	120
4	Hot Water 60 C	12.5	33.33	0.00	4.6	15	8	120
4	Hot Water 70 C	16.2	33.33	0.00	9.4	15	9	135
4	Hot Water 80 C	5	13.33	0.00	1.6	15	8	120
4	Hot Water 90 C	0.00	0.00	0.00	0.00	15	8	120
4	Hot water 100 C	0.00	0.00	0.00	0.00	15	8	120

Table 2-2. Statistical Results of Seed Tests.

Comparison of:	F or Chi Value	P	Pos Hoc	Significance
Controls	0.55 F	0.65		
Age	0.55 F	0.65		
Treatments Trial 1	5.98 F	0.0193	Sandpaper, 0.02	**
Treatments Trial 2	3.61 F	0.0161	HW70C, 0.03	**
Treatments Trial 3	5.798 Chi	0.220		
Treatments Trial 4	21.562 Chi	0.0105	Acid 1 min HW80C, 0.0275	**
Sandpaper vs 50C H W: Trial 1	6.69 F	0.0609		*
All Hot Water 50C	1.047 F	0.4		
All Hot Water 60C	3.6 F	0.0563	6 ^{mo} A,	
All Hot Water 70C	9.03	0.003		
All Hot Water 80C	1.44	.2577	Hw70C-A,	
All Hot water	4.1 F	0.002	HW70C-A	
All HW vs Control	3.66	0.001	HW 70C-A	
All Acid 1 minute	10.84	0.008	Acid 1 min-D	***
All Acid 2 minutes	0.68	0.4425		
All Acid 3 minutes	1.74	0.211		
All Acid 4 minutes	8.57	0.0049	Acid 4 min- 5mo	***
All Acid	1.94	0.11		
All Acid vs Control	4.00	0.031	Acid 3min, Acid 1 min.; 0.02	**

*(p=.1),**(p=.05), ***(p=.01)

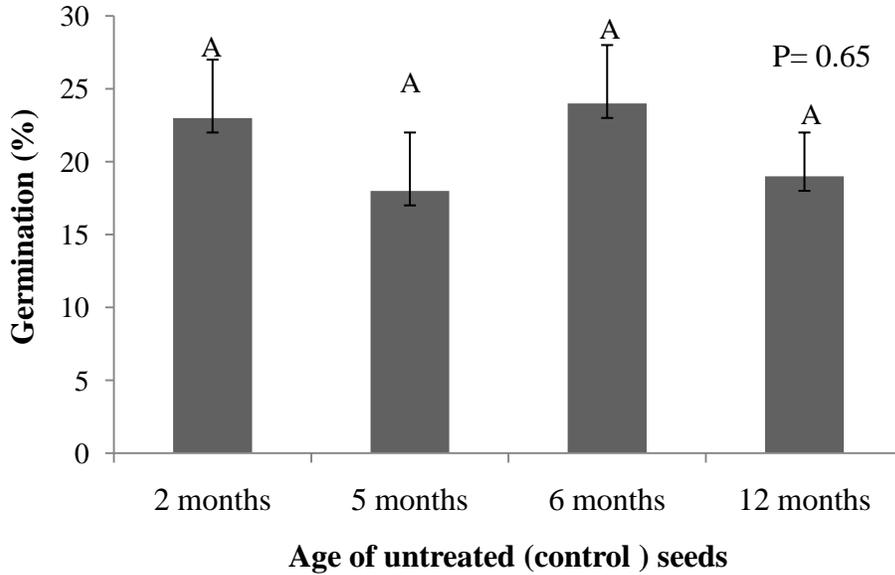


Figure 2-1. Effect of seed storage on germination; note that there is no difference in germination amongst the untreated (Controls) of the three trials. Error bars measure SE. (*n* per treatment=4 except for 5 month trial, where *n*=8 and 6 month trial where *n*= 3)

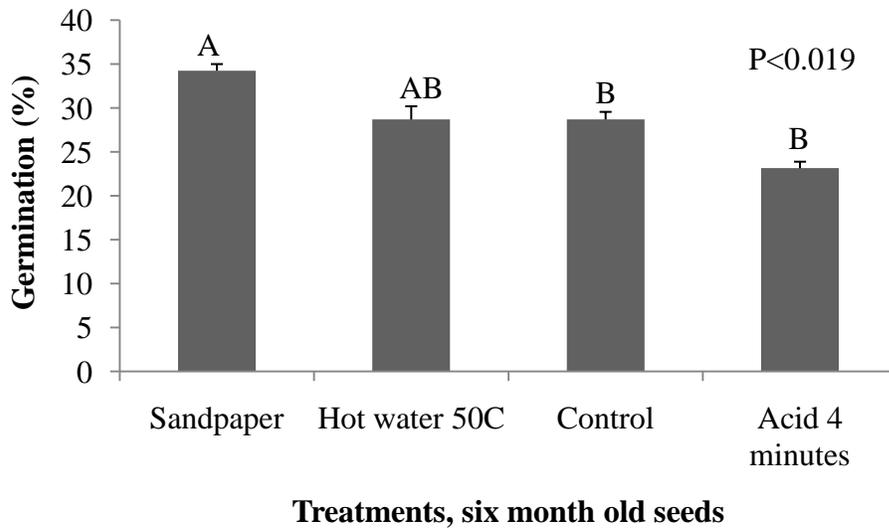


Figure 2-2. Results of first trial. Sandpaper is the physical scarification treatment. Error bars measure SE. (*n* per treatment=4)

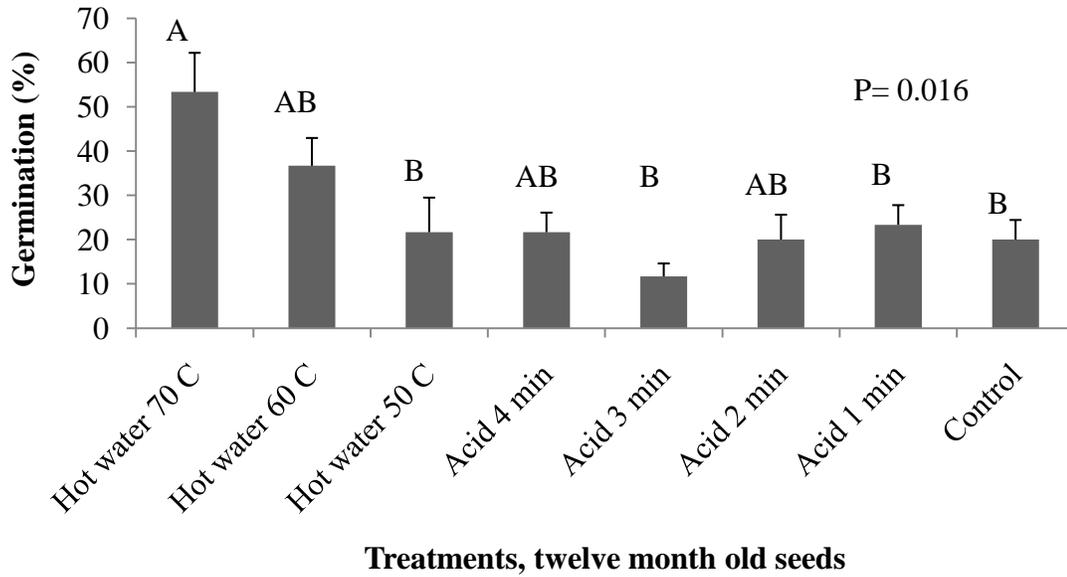


Figure 2-3 Results of second trial. Notice hot water 70 C treatment has best result; with average germination of 53%. Error bars measure SE. (n per treatment=4)

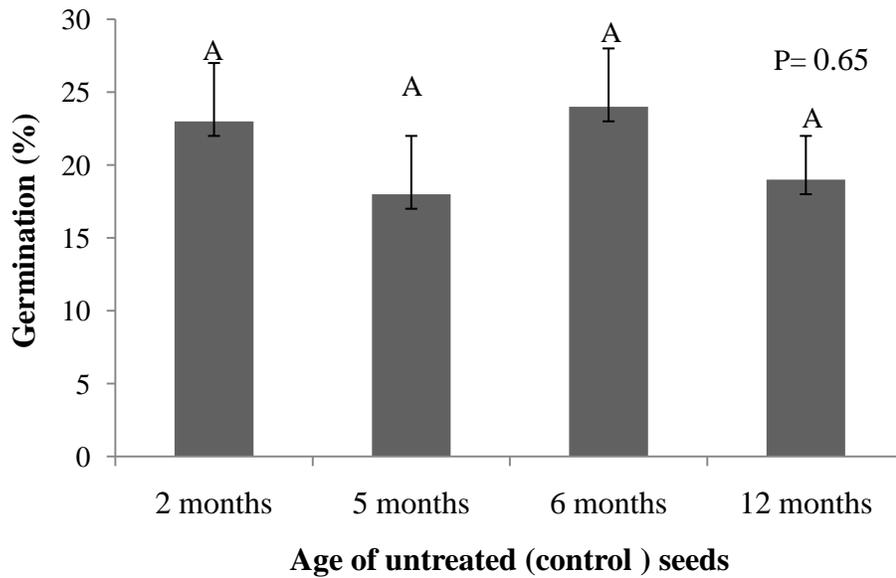


Figure 2-4 Results of third trial with 2 month old seeds. Note that control is highest treatment overall and that the 70 C Hotwater treatment is still the best hot water treatment. $P < .22$ if results from Hot water (0C data excluded. Error bars measure SE. (n per treatment=4)

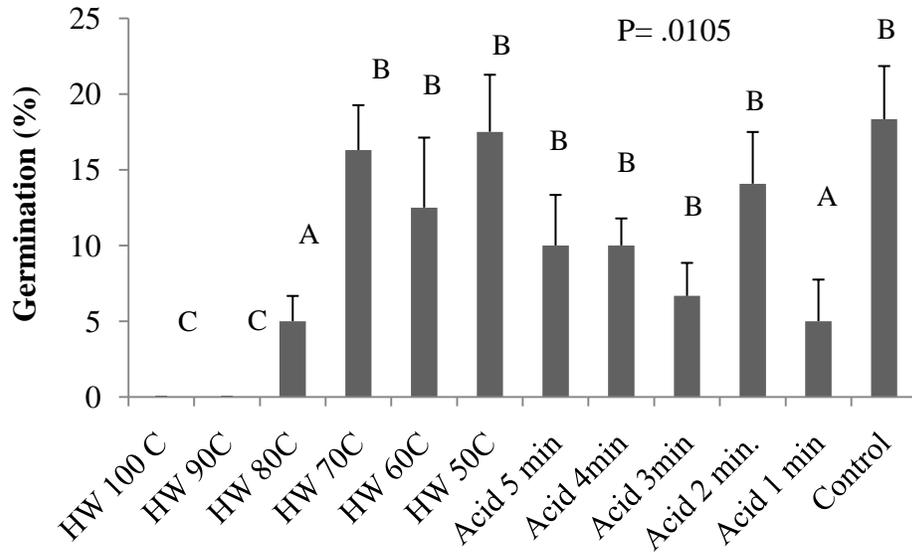


Figure 2-5. Results of fourth trial. Once again confirms that water at 90 C and 100 C kills seeds. Error bars measure SE (n per treatment=8)

CHAPTER 3
QUANTIFYING DROUGHT TOLERANCE OF *Bursera graveolens*

Introduction

Bursera graveolens, a tropical deciduous tree species, grows in Ecuador and Peru in areas with annual rainfall between 250 mm and 1000 mm (Valverde 1990). Specifically, it grows in tropical desert scrub, tropical thorn woodland and “*very dry*” tropical forests as well as subtropical dry forests and thorn woodland as defined by Holdridge (Sánchez et. al., 2006; Valverde, 1990, 1998; Holdridge, 1978). It is subject to severe water stress because the distribution of rainfall events in its Ecuadorian and Peruvian home range is extremely seasonal with a dry season lasting anywhere from six to eight months. *B. graveolens* is an early successional species with a commercially valuable fragrant wood. It colonizes disturbed, arid and nutrient poor sites (Guerrero & Lopez, 1993). While it is known that *B. graveolens* habitually loses its leaves during the dry season, the ecophysiological basis for its drought tolerance has not been examined.

Fresh water is a limited resource. Most tropical forests experience seasonal droughts that last for weeks or months, even in many classified as moist or humid (Mulkey & Wright, 1996). Forest plants also can suffer water limitation daily, during the heat of the midday or even from competition with other plants in the shade of the understory (Kainer, 1998).

Plants have various physiological responses and strategies in response to the availability of resources such as light, water, carbon dioxide (CO₂), and soil nutrients. Knowledge of the plant's responses to these resources is essential to understanding the plants survival and growth in a determined habitat (Rocha et al., 2006). Plants may tolerate or avoid drought. Drought tolerance is usually defined as the ability of cells to maintain physiological activity at low water level. These plants have high water use efficiency, that is, the ratio of CO₂ assimilation to transpiration.

Drought avoidance is a strategy by which desiccation of cells and tissue is avoided so that survival is ensured (Sinclair & Bennett, 1998).

Terrestrial vascular plants conserve water by having a cuticle covering the leaf and its photosynthetically active mesophyll, and possessing stomata that open and close. Stomata allow the intake of CO₂ for photosynthesis, while permitting the exit of water vapor and O₂. When light and water conditions are not limiting and biochemical processes are not impaired, photosynthesis occurs at maximum rates. If soil water is limiting, plants conserve water by reducing the size of the stomata opening, or if necessary, completely closing the stomata (Slayter, 1967). It is soil water potential, not leaf water potential as one might think, that affects the stomata, reports Schulze (1986).

Closure of the stomata inhibits gas exchange and thereby limits photosynthesis. When conditions become favorable again, such as increased soil moisture or reduced transpiration due to cooler temperatures, photosynthesis starts again. As long as stomata are still open, Farquhar and Sharkey (1982), found that stomata generally minimize water loss, relative to carbon assimilation.

Vascular plants try to conserve water they already have, store water and to obtain more. They have developed many mechanisms to do so. The most common mechanism is leaf loss in response to water stress. With no leaves, no water exits the stomata. It is documented that soybean plants (*Glycine max*, L. Merr.) when under drought stress reduce their exposure to sunlight by rolling over the leaves, exposing silvery pubescence (trichomes) on the lower leaf surface in order to reflect sunlight. Less sunlight leads to less stomatal opening and therefore lower water loss (Gardner et. al, 1985). Some tropical trees, such as *Bombax ceiba* L., store

water in their trunk and actually photosynthesis through their green, chloroplast laden bark during the dry season instead of using their leaves (Muthuchelian, 1992).

B. graveolens, and many of the trees that grow in association with it, possess traits for drought avoidance. It sheds its leaves at the start of the yearly dry season, as do many other associated species such as Guayacán (*Tabebuia chrysantha* (Jacq.) & G. Nicholson). Other trees are evergreen such as those in the family Capparidaceae. For example, *Capparistrum petiolare* has waxy leaves whereas *Colicodendron scabridum* (Kunth) Seem, possesses sclerophyllus leaves with pubescent undersides. Both traits reduce water loss through the stomata.

Another associate, the cascol (*Caesalpinia paipai*, Ruiz Lopez and Pavon), has tiny leaves which reduce the surface area exposed to evaporation. *Ceiba trichistandra* ((A.Gray) Bakh)), a tree closely related to *Bombax ceiba*, in addition to shedding its leaves during the dry season also has a green chloroplast laden trunk shaped like a bottle that stores water. In fact, local people, such as hunters, will cut roots as an emergency source of water (Yagual, 2003, *personal communication*). It is reported that two other members of the Burseraceae family, *B. microphylla* Gray and *B. hindsiana* Benth. Engler, both from the deserts of Baja California, are called as “tank” or cistern plants for the water that they store inside their stems (Scholander et al, 1965). Maybe *B. graveolens* stores water in its trunk as well.

Plants in arid areas also try to obtain more water, besides conserve the water they have. Sometimes belowground biomass is greater than above ground biomass (Gardner et.al., 1985). A greater proportion of biomass is dedicated to roots rather than stems and foliage. The more roots a plant possesses, the more soil there is to be exploited for water and nutrients. Roots represent 18 to 46% of the total biomass in tropical dry forests (Sanford and Cuevas, 1996). Some arid land species such as *Prosopis* spp. are also known for their very deep roots that will grow down

to deep subterranean water tables. This is the case with Algarrobo, *Prosopis juliflora*, (Sw.) DC), an acacia-like tree that grows in association with *B. graveolens*. It can extract water from water tables 15 m deep and may be even more than double that depth (FAO, 1992). Another member of the genus, *P. cineraria* L. Druce, has been reported to extend its roots as deep as 60 m below ground to tap into ground water in the Oman desert (FAO, 1992).

Besides water, CO₂ is the other raw material of photosynthesis. The carbon within the, CO₂ molecule is used for fueling cellular processes and building plant tissues. The entrance of CO₂ molecules into the leaf is regulated by the stomates. The stomates, in turn are regulated by soil water potentials and water vapor gradients between the leaf and the air (Schulze, 1986). Farquhar and Sharkey (1982), state that a reduction in stomatal conductance also leads to a reduction of CO₂ within the leaf's mesophyll and therefore a reduction in photosynthesis and growth. This reduction in stomatal conductance, often times if not exclusively, is caused by lack of water or drought.

Other investigators have questioned this (Manter & Kerrigan, 2004; Manter et al., 2000). They found unchanging or increasing levels of internal CO₂ (C_i) in spite of reduced stomatal conductance (g_s) with water stressed plants. They attribute the reduction of photosynthesis normally associated with drought not on reduced conductance, but rather on damage to the photosynthetic machinery of photosystems I and II. They call this non stomatal inhibition (Anderson et al., 1995). Other studies of leaf fluorescence show that leaf photochemistry is resistant to moderate drought stress. Finally, other investigators blame conflicting results on patchy stomatal closure. That is to say, not all the stomata on a leaf close at once or uniformly across the leaf surface (Sharkey & Badger, 1982).

According to Brodribb (1996), the important question to answer is whether nonstomatal

inhibition occurs early during drought, depressing photosynthesis in spite of significant rates of gas exchange, or whether this effect occurs only in highly stressed plant as stomatal conductance approaches the minimum. Obviously at some stage of drought, direct inhibition of photosynthesis will occur either due to turgor loss, cell damage, or loss of chemical functions in the chloroplasts.

The objective of this experiment was to determine and quantify the physiological responses to water stress of one-year-old *B. graveolens* seedlings. One year old nursery grown seedlings are what are typically planted in reforestation or restoration projects. The following questions were addressed: (1) How do seedlings allocate biomass when they are grown in a low water environment? (2) How do seedlings respond to drought in terms of gas exchange and plant water potentials? (3) Is *B. graveolens* a drought avoider or a tree that tolerates drought or shares features of both strategies?

Materials and Methods

The plan of this experiment was to progressively subject one-year-old *B. graveolens* seedlings growing in 7.6 liter pots to water stress. In order to do so, plants were raised from seed and transplanted at one month of age in September 2007 into plastic pots. The potting medium was a 50-50 mixture of commercially available sand (Quickcrete Premium Play Sand) and topsoil bought from a commercial garden nursery. Four measuring cups, (250 ml), of the top soil-sand mix were sent to a commercial lab for analysis of soil texture, pH and nutrient status. The mixed soil was composed of 94% sand, 3% clay and 3% silt particles. Soil pH averaged 7.5. The potting mix had no nutrient deficiencies at the moment of planting, although after 11 months, it was necessary to add granular plant fertilizer (Osmocote 19-6-12), at a dose of 4 grams per pot to compensate the nutrient deficiencies developed over time as per Svenson (1993) and Docherty et al.(1997), on May 19, 2008.

A month before the start of the experiment, all plants were kept well watered with soil water at field capacity. Field capacity is the percentage of water remaining in the soil after it has been saturated and after free drainage has practically ceased (Brady & Weill, 2002). During the duration of the experiment, plants in the well watered treatment received water every 3 days. The plants were irrigated by hand with a pitcher until water drained out of the bottom of the pot.

Overall, 60 seedling were arranged in a completely randomized block design with two blocks and two treatments. Plants were randomly selected to receive either a well watered treatment (WW) or water stressed (WS) treatment. Once the experiment started on October 18, 2008; the plants selected for the water stress treatment received no additional water until December, 28th, 2008 when the experiment ended.

The plants were grown and kept alive in a glass house at the University of Florida in Gainesville. Temperatures within the glass house never fell below 0°C and were not higher than 30°C. Day length was shortening from 12.5 hours at the start of the experiment on October 17th, to 10.5 at the end of the experiment on December 28th. Therefore, the photoperiod the plants were exposed to was similar to that of Ecuador at the start of the experiment, and noticeably shorter at the end. Sunlight was the primary source of illumination. Differences in sunlight intensity and day length between Gainesville and Ecuador were a concern, but it appears not to have affected the experiment. Maximum CO₂ assimilation values (A_{max}) values for the WW plants showed no statistically significant difference at the start of the experiment or at the end of it ($p = 0.21$).

Sampling was performed approximately twice a week over the course of 72 days. All plants had their photosynthetic assimilation, stomatal conductance, internal CO₂ and transpiration recorded when subjected to 2,000 $\mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$ of photosynthetically active

radiation (PAR) by the using of a *LI-6400* gas exchange system (*Li-Cor*, Lincoln, Nebraska). Chazdon et. al., (1996), cites maximum PAR values of $2224 \mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$ in the summer in the almost tropical city of Miami and PAR values of up to $2600 \mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$ in the canopy level of a tropical forest. It is also the maximum PAR setting on the *Li-Cor 6400*. The leaf chamber was set to an approximate ambient CO_2 concentration of $400 \mu\text{mol l}^{-1}$, and the air flow was maintained at $500 \mu\text{mol s}^{-1}$. Temperature of the IRGAS block, which contains the leaf chamber, was set to 25°C . Before measurements were taken, the leaves were photosynthetically induced for 15 to 30 minutes so that photosynthetic values stabilized before a reading was taken. All measurements were taken between the hours of 0800 and 1600.

In addition to measuring the photosynthetic capacity of all sampled plants at $2000 \mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$ of PAR, a light curve was determined on one water stressed plant and one well watered plant per block every week. The light curve measured the photosynthetic response of the plants to varying light intensities and decreasing soil moisture. Light settings progressively increased in steps from $0 \mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$ to $2000 \mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$. The light curve data demonstrated that maximum CO_2 assimilation occurred at $2000 \mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$ and not at some lower light level. The third fully expanded leaf down from the top was selected for gas exchange measurements. If that leaf was torn, chlorotic, or showed signs of insect or fungal damage, the next available leaf was chosen.

Both pre-dawn and midday leaf water potential were measured with a pressure chamber (Boyer, 1995; Scholander et al, 1965). The same leaf chosen for the measurement of photosynthesis was used for the midday water potential. Since measuring leaf water potentials with a pressure chamber destroys the leaf, a different but similar leaf from that used to measure photosynthesis was chosen for the measurement of dawn water potentials.

Gravimetric soil water content was determined for each pot from a sample of approximately 50 g of soil every time a measurement of photosynthesis occurred. The moist soil was first weighed, and then dried at 105°C in an oven for 24 hours to calculate the bulk density. With the moist weight and the dry weight of the soil known, water weight and water volume was determined for each pot (Brady & Weill, 2002).

Water stressed plants were harvested, once they lost all of their leaves. The well watered seedlings and those water stressed seedlings that still possessed leaves, were harvested on 28 December, 2008 and separated into their component parts: leaves, stems and roots. Plant components were and dried at 65°C before determining biomass. Individual component biomass, total biomass, and root and shoot ratios were calculated. Root water content was calculated by the formula:

$$\text{Root Water Content} = 1 - (\text{dry weight/fresh weight}) * 100. \quad (3-1)$$

Data Analysis

The data was analyzed by ANOVA within the framework of a randomized complete block design. Tukey's post hoc tests were used for pair wise comparisons and differences between means were declared significant at $\alpha < 0.05$. The statistical program JMP-IN 5.1 for Windows (SAS Institute, Cary, NC, USA) was used to perform the analyses.

Results

No differences were found between blocks, but differences were found between treatments. Total mean biomass production was significantly higher for seedlings in the WW treatment; ($p > 0.003$). If the biomass production is calculated including the foliage, the WW seedlings were 35% larger than WS seedlings, but only 20 % larger if foliage was not taken into account (Table 3- 1, Figure 3- 1, Figure 3-2.). By the end of the experiment, the WS plants had lost or were losing their leaves while the WW plants kept their foliage. This is not an unusual

reaction to water stress on the part of *B. graveolens*, because it habitually loses its leaves during the dry season. Foliage made up for 11 % of the biomass of the WW seedlings and less than 1% of the WS seedlings. Therefore, foliage was excluded from some biomass calculations.

The WW seedlings contained 31% more shoot biomass (46.4 g), compared to the WS seedlings (35.5g). Root biomass was also 3% greater in the WW (23.7 g), plants compared to the WS plants (23.0 g). However, the WS plants allocated proportionately more biomass to roots; 38% compared to 29% for the WW plants ($p = 0.0007$), resulting in a significantly higher root: shoot ratio for this treatment. The root: shoot ratio of WS seedlings was 0.65 compared to 0.51 for WW seedlings ($p = 0.0011$) (Figure 3-3). The water content in the fresh roots was found to be 52 % of the root biomass for the WS plants and 46 % of the root biomass for the WW plants. After 72 days without watering, 57 % of the WS plants still retained some leaves (Figure 3-4).

Soil water decreased over time. The WW plants had an average soil water content of 19% through the duration of the experiment. Water stressed plants started with soil with a similar soil water content of 18% and finished with an average of 2% soil water content if they still had leaves (Figure 3-5). Plants that had all shed all their leaves were assumed to have less than 2% gravimetric soil water content.

Predawn leaf water potential was strongly correlated with soil moisture content in WS and WW plants. It is a statistically significant trend when graphed: $r^2=0.35$ (Figure 3-6). 35% of the variability in predawn leaf potential is explained by soil water content. Midday leaf water potentials averaged - 0.4 MPa for the WW plants and the WS plants averaged -1.1 MPa with minimum MPa being -1.4. However, it is not as strong a trend as gravimetric soil moisture plotted against time which has an R^2 equal to 0.61.

As gravimetric soil water decreased over time, so did assimilation of CO₂ at the light saturation point, (A_{max}), stomatal conductance, (g), mesophyll CO₂, (C_i), and transpiration, (T), in all the WS plants. Assimilation of CO₂ by seedlings in the WS treatment occurred over a wide range of soil moisture levels, and showed a high range of variability before decreasing, and then ceasing. As mentioned earlier, photosynthesis, and by extension, assimilation of CO₂ came to a halt, when gravimetric soil water content fell below 2%, and the leaves fall off the plant. In summary, there is a downward trend of decreasing A_{max} with decreasing gravimetric soil water content. However, the correlation value is very low ($R^2=0.04$) and it is not statistically significant.

A_{max} averaged $9.1 \mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$ for seedlings in the WW treatment, and was $7.1 \mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$ in for seedlings in the WS treatment. Stomatal conductance, (g), transpiration, (T), and intercellular CO₂ within the mesophyll, (C_i), all showed a statistically significant declines as soil water decreased with strong regression coefficients that were statistically significant ($r^2=0.2, 0.21, 0.1$) ($p = 0.001, 0.011, .027$). WS plants had a mean C_i value of $136 \mu\text{l}^{-1} \text{m}^{-2}\text{s}^{-1}$ and WW plants had a mean C_i value of $254 \mu\text{l}^{-1} \text{m}^{-2}\text{s}^{-1}$.

The values of these photosynthetic variables stayed more or less the same for plants in the WW treatment throughout the duration of the study. These plants had an average gravimetric soil water content of 19% and never experienced water stress. However, individual readings of gravimetric soil water content for the WW plants varied from 14% to 24% (Table 3-2, Figures 3-7 and 3-8).

In fact, plants growing in soil with soil moisture content over 21 % appeared to have a decline in (A_{max}), (g), (T), and (C_i). This suggests that the soil was too wet for optimal CO₂ uptake and growth for *B. graveolens* and that the species will not grow well on soils that

sometimes flood and become water saturated. Even in arid areas, clay soils or soils overlying an impermeable layer can become waterlogged immediately after an infrequent but intense rainstorm.

The average light curve data showed that A_{max} was statistically similar for plants growing in soil moisture levels of 19, 15 and 10% with mean A_{max} of $8.26 \mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$. Their standard errors tended to overlap. Whereas, the A_{max} value for plants growing in soil with a gravimetric water content of 2% was $4.94 \mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$. Their standard errors did not overlap with the plants growing in soil with gravimetric water content of 8% or above, ($p > 0.0007$) (Figure 3-9).

Discussion

How do seedlings allocate biomass when they are grown in a low water environment? Sanford and Cuevas (1996), state that between 18% and 46% of the biomass in tropical dry forests consists of roots. They write that tropical dry forests have more biomass in soil than tropical humid forests. The exceptions are tropical forests growing on spodosols or mangrove forests, where below ground biomass exceeds aboveground biomass. In this experiment, the percentage of *B. graveolens* biomass (not including leaves), allocated to roots was 38 and 33% for WS and WW plants, respectively. These two values appear reasonable and fall within the range cited by Sanford and Cuevas (1996). These values also appear within the range of values reported by Celis-Azofeifa (2006), for two species that grow in association with *B. graveolens*; *Tabebuia rosea* (Bertol.) DC., and *Pseudosamanea guachapele* (Kunth). The first species, *T. rosea* had an average root mass percentage of 20% and *P. guachapele* had 50% for nursery grown plants.

The WS seedlings of *B. graveolens* had proportionately more root biomass (14% more) than the well-watered seedlings and it was statistically significant at $p=0.001$. In a study by Jose et al. (2003), dealing with *Pinus palustris* Mill., water stressed plants produced proportionally

more roots than abundantly watered plants. They reported a 69% increase in the root: shoot ratio for nutrient stressed plants and a 19% increase in the root: shoot ratio for plants that were only water stressed. Runion et al. (1999) found that nutrient stress and water stress in *Pinus palustris* seedlings increases the root: shoot ratio of 41% and 14% respectively. My result of a 14% increase in the root: shoot ratio for water stressed seedlings of *B. graveolens* appears reasonable, although a more in depth statistical analysis of the three studies would be needed to confirm this. It is less than Jose's 19 % increase in root: shoot ratio for water stressed individuals of the temperate species, *P. palustris*, but the same as found in Runion et. al (1999).

The *B. graveolens* plants in this experiment were not chlorotic, and believed not to be under damaging nutrient stress. They were fertilized prior to the start of the experiment to replenish soil nutrients lost after a year's growth. Therefore, only the affects of water stress and the absence of water stress could be compared.

Henderson and Jose (2005) found that increased soil water did not necessarily lead to increases in tree biomass; it often had to be accompanied by increase in nutrients. This experiment with *B. graveolens* confirms that water stress decreases total plant biomass. It would be interesting to see how *B. graveolens* reacts to nutrient stress because, in general, the species grows on nutrient poor sites or sites with little topsoil. An experimental treatment combining water and nutrient stress would mimic typical field growing conditions.

It is known that *B. graveolens*, loses its leaves during the yearly dry season, becomes dormant only to put out flower and put out leaves at the start of the rainy season. Another interesting experiment to run would be to see how the growth of *B. graveolens* reacts to limited water stress; i.e., the water stressed plants periodically receive water throughout the duration of the experiment so that leaf fall is not induced, but does not receive as much water as those

plants in the well watered treatment. How do the leaves recover to temporary drought stress? Is there damage to the photosynthetic machinery?

How do seedlings respond to drought in terms of gas exchange and plant water potentials? *B. graveolens* can be considered an early successional species., Several investigators have reported that the maximum assimilation of CO₂ at light saturating conditions or A_{max} for pioneer trees (early successional species) growing in high light conditions range from 11- 22 $\mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$ (Davidson et al., 2002; Holscher et.al, 2004; Tinoco-Ojanguren & Pearcy, 1995). *B. graveolens* has A_{max} values within these ranges, particularly if the plant is in moist soil at field capacity. However, the potential maximum rate of CO₂ assimilation is not often reached due to limiting resources such as light, nutrients, water, herbivore damage and internal feedback mechanisms (Bazzaz, 1996).

A_{max} for *B. graveolens* in this experiment range from a minimum of 0.274 $\mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$ for a severely water stressed plant at 2% soil moisture content to a maximum of 21.3 $\mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$ for an abundantly watered plant, with a soil water content of 18 %. Average A_{max} is 9 $\mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$ over a broad range of soil moisture conditions. A table of A_{max} values cited by Strauss – Debenedetti and Bazzaz (1996), report early successional species taking up CO₂ in the range of 5.5 to 27 $\mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$. The A_{max} values for WW seedlings with a gravimetric soil water content of between 14% and 24% appear to be within the ranges of values achieved for other neotropical early successional tree species. For example, *Cordia alliodora* (Ruiz and Pav) Oken), is a fast growing, long lived, early successional timber species that grows in association with *B. graveolens*. It has been reported as having CO₂ assimilation of 6 $\mu\text{mol}^{-1} \text{m}^{-2}\text{s}^{-1}$ in 100% sunlight or full sun. *Brosimum alicrastrum* Sw., a late successional tree species that also grows in

association with *B. graveolens*, is cited as possessing a CO₂ assimilation rate of 6 μmol⁻¹ m⁻²s⁻¹ in full sun (Strauss-DeBenedetti & Bazzaz, 1996).

Kitajima (1994) documented early successional and shade intolerant seedling of species that grow in the same area of *B. graveolens* such as *Cavanillesia platanifolia*, *Ceiba* spp., *Cordia alliodora*, *Ochroma pyramidale*, as having CO₂ assimilation values between 5 and 10 μmol⁻¹ m⁻²s⁻¹ at 800 μmol⁻¹ m⁻²s⁻¹ of PAR. Although this study does not have CO₂ assimilation values taken at 800 μmol⁻¹ m⁻²s⁻¹ of PAR, there is data available from the light curves at 700 μmol⁻¹ m⁻²s⁻¹ of PAR. CO₂ assimilation for *B. graveolens* is between 6 and 7 μmol⁻¹ m⁻²s⁻¹ for plants not subjected to water stress. However, owing to the fact that different species vary in leaf thickness, it is suggested that A_{max} value should be compared by unit mass as opposed to unit area (Kitajima, *personal communication*). The leaves of *B. graveolens* are much thinner when compared to the leaves of *Brosimum alicastrum* (Morgan, *personal observation*).

Two different studies performed upon the congener *B. simaruba*, place CO₂ assimilation in full sunlight at 11.8 μmol⁻¹ m⁻²s⁻¹ and 13.9 μmol⁻¹ m⁻²s⁻¹ respectively, (Chazdon, 1997, Strauss-DeBenedetti and Bazzaz, 1996). In the experiment described here, *B. graveolens* seedlings were recorded with CO₂ assimilation values as high as those achieved for *B. simaruba* or higher.

The interesting finding of this experiment, however, is that A_{max} for *B. graveolens* remains constant over a wide range of gravimetric soil moisture values. A_{max} was statistically the same for plants in soils with 8 to 21 % soil moisture content. At the start of the experiment, the average gravimetric moisture content for the soil in the pots was 19%. Photosynthetic capacity significantly declined when the gravimetric moisture content of the soil fell below 8%, but was still relatively high at 2%, with an average A_{max} of 4.94 μmol⁻¹ m⁻²s⁻¹. It should be mentioned

that after 72 days, 57% of the water stressed plants still possessed foliage at gravimetric soil water content of 2 %.

Once the gravimetric soil water content falls below this threshold of 2 %, the leaves fall off the seedlings of *B. graveolens* and photosynthesis ceases, as far as we know. There are two reasons for this, either the leaves fell off the seedlings or the plants were reduced to possessing two diminutive leaves on the apex of the stem. These tiny leaves were too small to measure photosynthetic gas exchange with the LI 6400.

Photosynthesis might still be carried out by chloroplasts under the bark of young branches (Muchelian, 1992; Pfanz et al, 2002). Perhaps water stored in the roots or tissues of the stem, allow the trees to photosynthesize, maintain a positive or at least neutral carbon balance, and stay alive. With that in mind, we measured the water content of the roots immediately after whole plant harvest.

The mean water content in fresh roots of *B. graveolens* was found to be 52% for the WS plants and 46% for the WW plants. These values are well below those found by Slot and Poorter (2007). They studied four tree species found within Bolivian tropical dry forests. They report that root water content varied between 62 and 84% for plants at full turgor and conclude that in order to tolerate drought, it is more useful to store water in the roots than have a longer root length to explore a drying soil. However, the *B. graveolens* seedlings subjected to the WS treatment still had an average root water content of 46%, when the gravimetric soil water content was 2%. One would expect that the *B. graveolens* seedlings would have similarly high root water content when compared to the Bolivian *dry* tropical forest species; not a lower water content. This is surprising, especially for the *B. graveolens* seedlings in the WW treatment, which could be considered at full turgor.

The leaf water potential data for *B. graveolens* seedlings in the WS treatment is comparable for other tropical dry forest trees that are deciduous. This experiment found that past -1.4 MPa and 2% gravimetric soil water content, *B. graveolens* loses its leaves. Olivares and Medina (1992) measured leaf water potential of several tropical dry forest tree species in a Venezuelan *very dry* tropical dry forest with almost identical annual precipitation as the Cerro Blanco protected forest. They state that reduction in soil water availability appears to be the main factor inducing leaf drop in deciduous trees. Some of the same species grow in association with *B. graveolens* in Cerro Blanco (Morgan, *unpublished data*). They are the drought deciduous *Tabebuia chrysantha* (Jacq.) and G. Nicholson, the (almost) evergreen *Sapindus saponaria* L. and the evergreen *Capparis flexouosa* (L.) L.. They also studied the congener *B. simaruba*, which occurs in Venezuela, but not in Ecuador. Both *T. chrysantha* and *B. graveolens*, lose their leaves at a similar leaf water potentials; -1.5 MPa versus -1.4MPa. The two evergreen species reached zero turgor at -2.14 MPa for *S. saponaria* and *C. flexuosa* at -3.79 MPa. *B. simaruba* reaches zero turgor, and then leaf loss occurs at -0.9 MPa. It appears that *B. simaruba* is not as drought tolerant as *B. graveolens*.

Scholander et. al. (1968) measured leaf water potentials of two *Bursera* species growing in the deserts of Baja California; *B. hindsiana* (Benth) Engler, and *B. microphylla* A. Gray. Leaf water potentials of the two congeners ranged between -0.68 and -1.5MPa. They made the following observation: “The suction of these extreme desert plants was moderate. It had rained one month earlier, and undoubtedly, like cacti, these plants avoid excessive tension by storing water. Presumably, the leaves are shed early before water potential has dropped excessively.” These figures appear to be similar to *B. graveolens*, which sheds its leaves early in the dry season as well.

In another study by Rocha et al. (2006), the tropical tree, *Miconia guianensis* Aubl., closes its stomata and ceases photosynthesis after 35 days of drought and once a mean leaf water potential of -3.18 MPa is reached. The tree is from the central Amazon and not deciduous. Although it grows in what is considered a humid tropical forest with above 2000 millimeters of precipitation annually, it also is subjected to a 5 month dry season between the months of June and November. Incidentally, this tree is in the Oleaceae family, that of the olives.

Olea europaea L., is a tree species originally grown in the Mediterranean Basin where the climate is characterized by hot, dry summers and cool, wet winters. Clemente, et al. (2006) performed an experiment upon 3 evergreen or sclerophyllous shrub species used for the restoration of limestone quarries in Portugal. One of these, *O. europaea* had a leaf wilting point water potential of -5 MPa. Like *O. europaea*, *B. graveolens* grows in an area of hot dry summers and grows on limestone outcrops. Instead of growing in a region of cool, wet winters, it grows in one of hot, wet winters. However, both *B. graveolens*, and the *O. europaea* trees used in the Portuguese quarry restoration project, are growing on limestone. Limestone outcrops are arid sites, because they have thin soil layers if any. Also limestone rock is porous and does not retain water. Unlike the sclerophyllous, drought resistant woody species cited above, *B. graveolens* sheds its leaves much before those leaf water potentials are reached. It loses its leaves to water stress at -1.4 MPa, not -3 or -5 MPa. However, the evergreen dry forest tree, *C. flexuosa*, of Olivares and Medina's study does lose their leaves at around -4 MPa.

Is *B. graveolens* a drought avoider, or drought tolerant, or a plant that shares features of both strategies? It is definitely a drought avoider because of its physical adaptations to drought such as deciduous leaves, a high root: shoot ratio of water stressed plants have compared to well watered plants and evidence of water storage in roots. The evidence that A_{max} levels remain high

over a wide range of soil moisture levels, and that photosynthesis occurs even at the low gravimetric soil water content of 2% suggests that *B. graveolens* is also drought tolerant.

Conclusion

B. graveolens increases root biomass relative to stem biomass in response to drought. It is a species that avoids water stress by storing water in its roots and shedding its leaves early, before water tensions rise to dangerous levels within in the xylem. *B. graveolens* maintains a positive carbon balance down to the low soil water content of 2 %. Furthermore, it maintains a relatively high rate of photosynthesis from gravimetric soil water content levels between 19% and 8 %. There is no statistical difference in the assimilation of CO₂ at these soil moisture levels. Below 8% soil water content, assimilation decreases and then ceases once soil water falls below 2% and the leaves fall off.

The relatively high rate of CO₂ assimilation by *B. graveolens* at higher soil water content levels point to possible use in mine land restoration. Supplemental irrigation can increase tree growth in the early years of seedling establishment. Once trees reach acceptable heights, they can grow and survive on site without supplemental irrigation. Positive CO₂ assimilation at low levels of soil water, in addition to root water storage, and the shedding of leaves at the onset of water stress allows the tree to grow and survive on arid sites. These results merit further rigorous investigation, ideally in tropical field conditions.

Table 3-1. Mean (\pm SE) of biomass data for each treatment. ($n = 30$).

Variable	Water Stressed	Well Watered
Total Biomass (g)	58.9 (1.7)	79.5 (4.0)
Leaves (%)	0.8 (0.2)	11.3 (0.6)
Stem (%)	60.7 (1.1)	59.1 (1.0)
Roots (%)	38.5 (1.1)	29.6 (1.0)
Stem biomass per total excluding leaves (%)	61.2 (1.1)	66.7 (1.1)
Root biomass per total excluding leaves (%)	38.8 (1.1)	33.3 (1.1)
Root: shoot ratio, excludes leaves	0.65 (0.03)	0.51(0.02)
Root water Content (%)	0.46 (0.01)	0.52 (0.01)

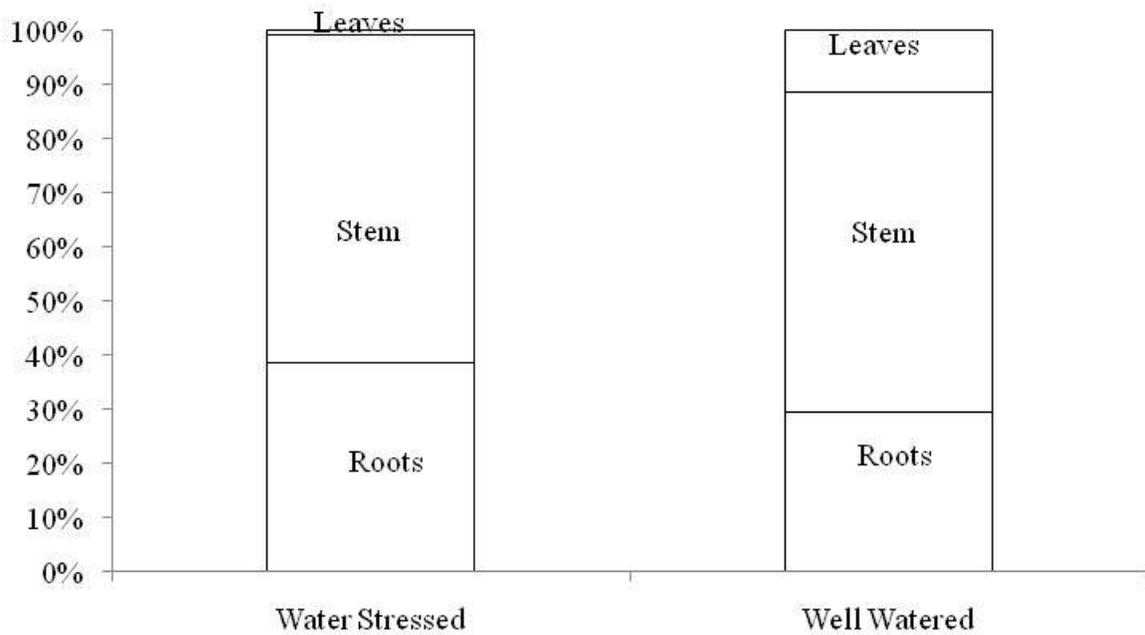


Figure 3-1. Graph comparing biomass of water stressed seedlings versus well watered seedlings. ($n = 30$).

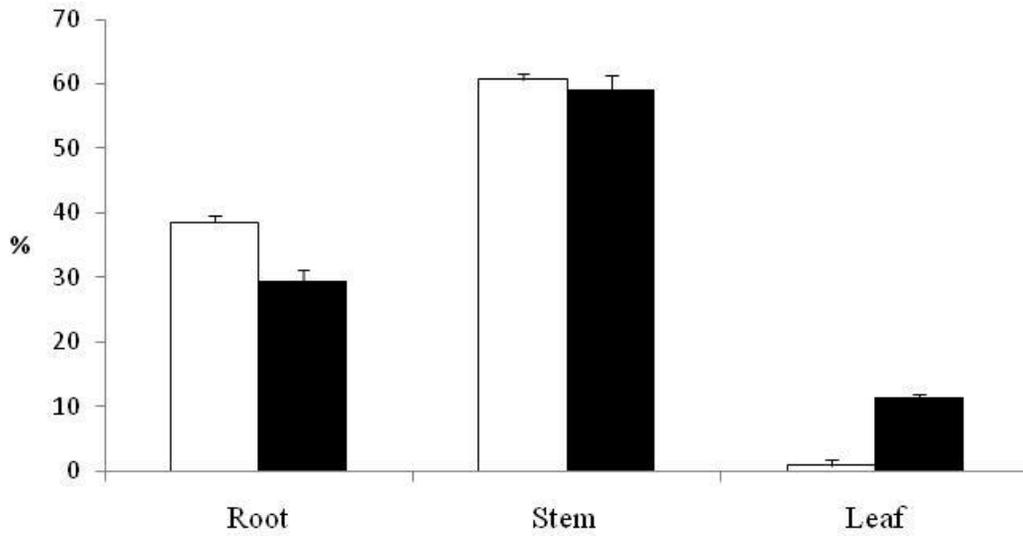


Figure 3-2. Graph comparing relative proportions of plant components. White bars are the water stressed plants and black bars are the well watered plants. Error bars represent SE. ($n = 30$).

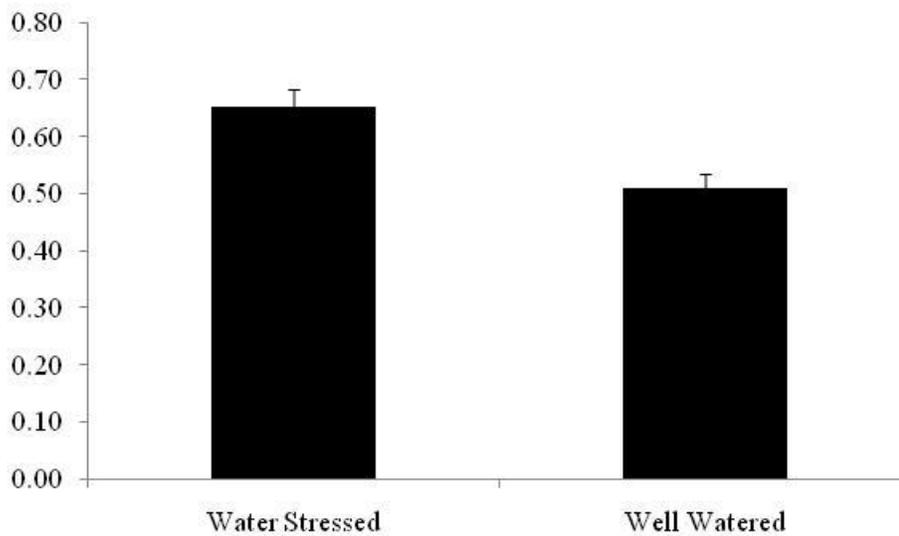


Figure 3-3. Graph comparing the root: shoot ratio of water stressed plants to well watered plants. Excludes leaves from calculation ($n = 30$ per treatment).

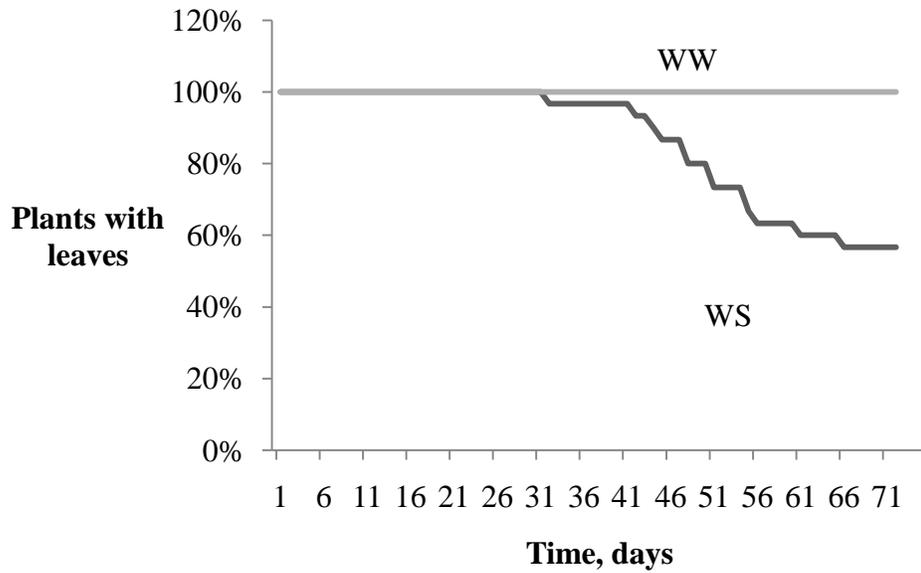


Figure 3-4. Leaf loss plotted over days. WW plants versus WS plants. ($n = 30$ per treatment).

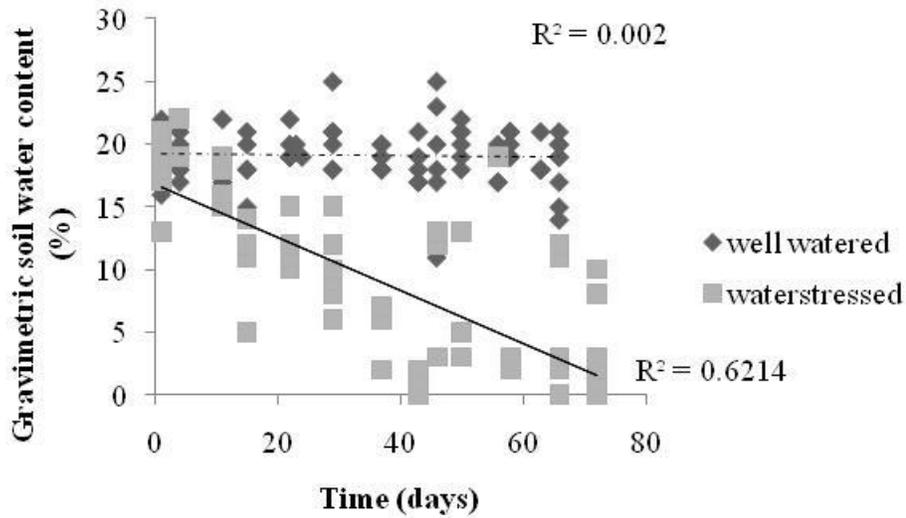


Figure 3-5. Soil water content plotted over days. ($n = 30$ per treatment).

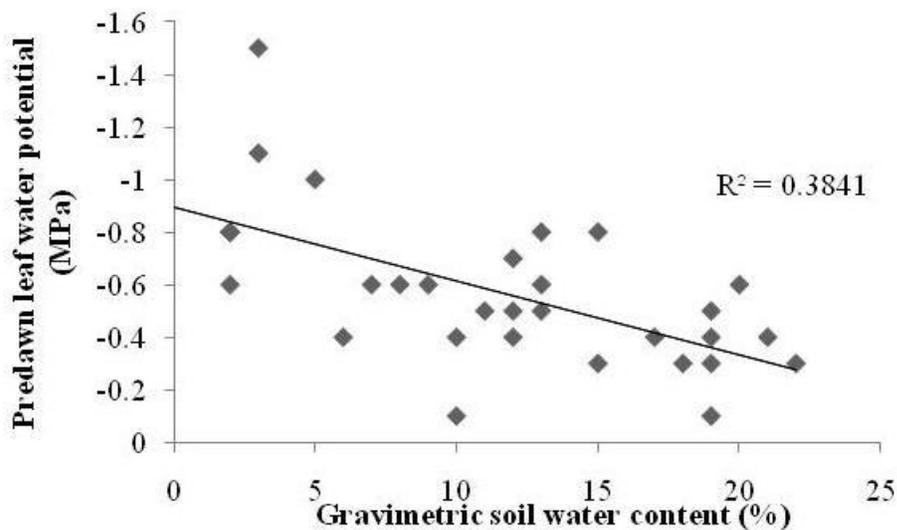


Figure 3-6. Predawn water potential plotted over soil water content. ($n = 30$).

Table 3-2. Mean (\pm SE) for photosynthetic variables for water stressed and well watered plants

Variable	Water Stressed	Well Watered
Assimilation of CO ₂ ($\mu\text{mol}^{-1}/\text{m}^{-2}/\text{s}^{-1}$)	7.1 (1.4)	9.1 (0.8)
Stomatal Conductance ($\text{mmols}^{-1}/\text{m}^{-2}/\text{s}^{-1}$)	0.04 (.01)	0.13 (0.01)
Mesophyll CO ₂ ($\mu\text{l}^{-1}/\text{m}^{-2}/\text{s}^{-1}$)	136 (28.6)	254 (11.8)
Transpiration ($\text{mol}/\text{m}^{-2}/\text{s}^{-1}$)	0.7 (0.2)	2.1 (.1)
Midday Water Potential (MPa)	1.1 (0.03)	0.4 (0.03)
Predawn Water Potential (MPa)	0.8 (0.3)	0.3 (0.03)
Soil Water Content (%)	3.0 (0.3)	19.0 (0.2)

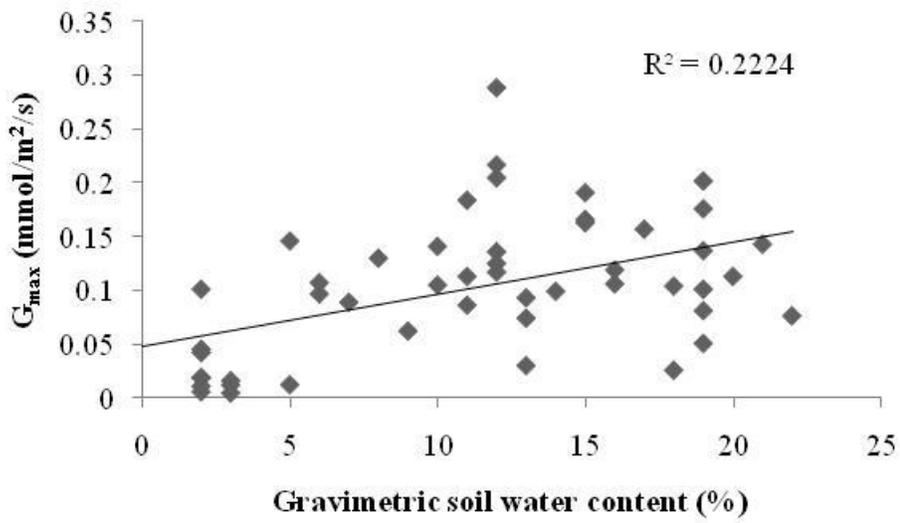
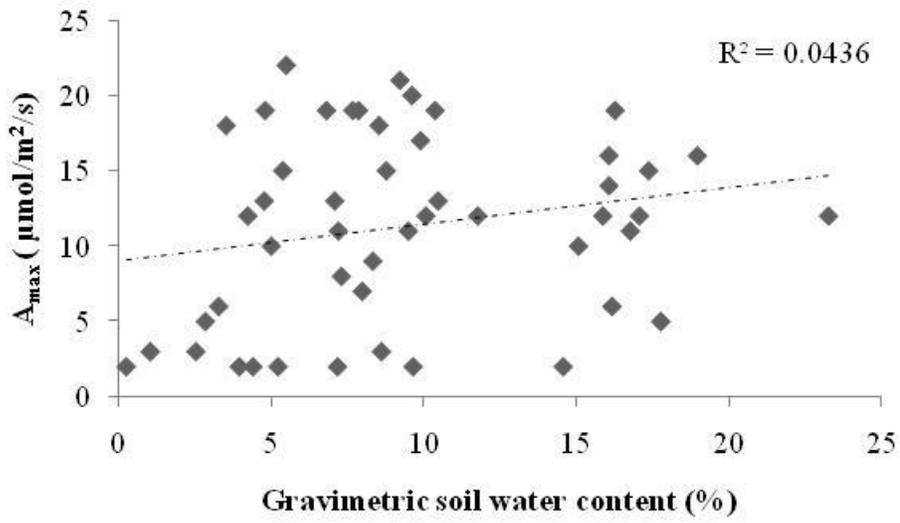


Figure 3-7. Assimilation and stomatal conductance for WS Plants plotted over soil moisture. ($n = 30$).

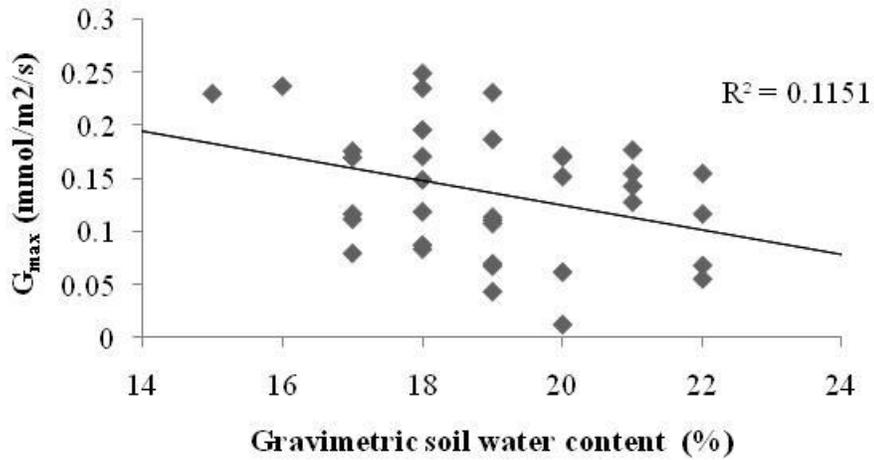
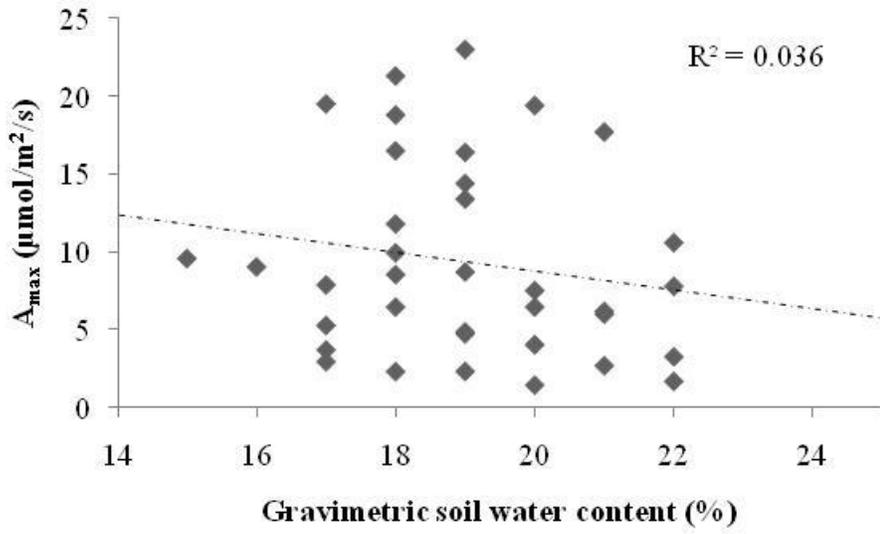


Figure 3-8. Assimilation and stomatal conductance plotted for WW plants. Notice that soil water content is between 14 and 24%. ($n = 30$)

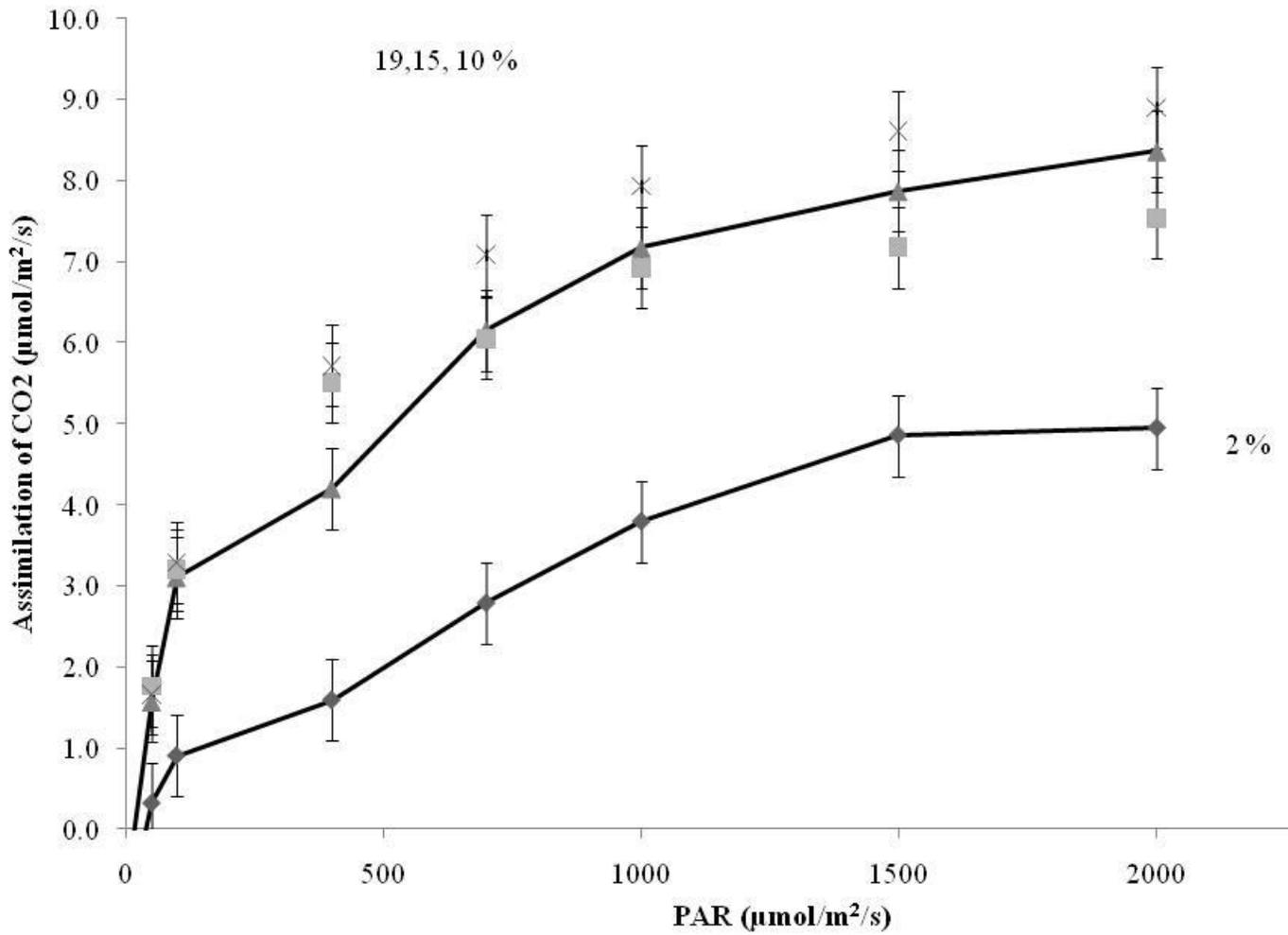


Figure 3-9. Values of average CO₂ assimilation values plotted against at differing levels of PAR for plants in 2 broad categories of gravimetric soil water content. Those plants as 19, 15 and 10% versus those at 2% . (n=24)

CHAPTER 4 SUMMARY AND CONCLUSION

Bursera graveolens (Kunth) Triana & Planch, locally known as Palo Santo is a small tree of Ecuador, Peru and other parts of the Americas. It is a member of the *Burseraceae* family that includes the *Boswellia* and *Commiphora* genera, which produce the raw materials used for the historically important incenses: frankincense and myrrh. *B. graveolens*' fragrant and resinous wood is highly sought after, potentially putting the tree in danger of extinction. The tree grows on arid and nutrient poor sites. The fragrant wood and the tree's drought hardiness suggest an aptitude for use in projects of ecological restoration in areas that once supported tropical dry forest and commercial potential for plantations dedicated to the production of resin.

I became interested in *B. graveolens* when I worked in the Cerro Blanco protected forest of Guayaquil, Ecuador from 1995 to 2006. I managed the tree nursery and worked producing seedlings of tropical dry forest trees for the restoration of abandoned pasture and abandoned quarry terraces. *B. graveolens* is a highly sought after species, however supply never could meet demand. The reason being was the poor level of seed germination in the nursery, about 8 %.

One of the missions of the foundation that employed me was to comply with Ecuadorian law and restore mining sites after exploitation. In this case, it meant abandoned limestone quarries to their previous condition of tropical dry forest. *B. graveolens* colonized old quarry terraces and other rocky sites without assistance, but colonization was slow. These sites are characterized by well drained, nutrient poor soils, high levels of sunlight, in addition to a dry season lasting from 6 to 8 months depending on the area. This is a very hardy tree. In order to better understand how the tree could be used in restoration projects, it was necessary to learn something about the physiology of the tree. The second part of this thesis was subjecting some

year old seedling to drought and measuring various photosynthetic variables and soil water content.

Worldwide, tropical dry forests and woodlands are characterized by precipitation between 250 mm and 2000 mm a year, and dry season lasting several months (Holdridge, 1978). It is the subtropical and tropical dry forests of the world that are quickly disappearing under human pressure. According to Janzen (1988), the tropical dry forests is the tropical lowland habitat most threatened with destruction; not the tropical rainforest. In Ecuador, less than 1% of the original dry forest currently exists (Sánchez et al, 2006; Neill & Nuñez, 1996).

While found at sea level near the Equator, the tree can be found growing at elevations as high as 1500 meters above sea level, particularly in the Andes of southern Ecuador and northern Peru (Colter & Maas 1999, Sánchez et al., 2006). More specifically, *B. graveolens* grows on rocky, arid, and nutrient poor soils (Guerrero & López 1993, Clark & Clark, 1981).

The seed of *B. graveolens* is covered in a fleshy red aril. The aril is consumed by birds that either consume the hard seed or excrete it. Many seeds need to be treated in some manner so that the seed coat becomes permeable to air and water, allowing germination. It was thought that by imitating the scarifying that occurs when a seed passes thorough the digestive tract of a digestive tract of a bird. After four different trials using hot water at different temperatures, brief acid soaks, and sand paper scarifying, the sand paper treatment worked best, followed by the untreated seeds of the control treatment control. Unfortunately, the sandpaper treatment is too labor intensive to be very practical on a nursery level. The control treatments gave an average germination of 22%, which was a higher rate of germination than obtained in Ecuador. It appears that simply storing the fresh seeds for minimum of two months allows the seed to dry increases germination, nearly tripling it. However germination is still unacceptably low and a test

with fresh seeds needs to be performed to confirm this. Also, perhaps, birds are less agents of seed scarification and more like agents of dispersal.

The seedling physiology study uncovered the surprising result that *B. graveolens* maintains a positive carbon balance down to the low gravimetric soil water content of 2 %. Furthermore, it maintains a relatively high rate of photosynthesis from soil water content of 19% to 8 %. This is comparable to other fast growing, early successional, tropical tree species. There is no statistical difference in the assimilation of CO₂ at these soil moisture levels. Below the gravimetric soil water content of 8%, CO₂ assimilation decreases and then ceases once soil water falls below 2% , and then the leaves fall off the plant.

The relatively high rate of CO₂ assimilation by *B. graveolens* at higher soil water content levels point to possible use as a tree for use in mine land restoration. Supplemental irrigation can increase tree growth in the early years of seedling establishment. Once trees reach acceptable heights, they can grow and survive on site without supplemental irrigation. Positive CO₂ assimilation at low levels of soil water, in addition to root water storage, and the shedding of leaves at the onset of water stress allows the tree to grow and survive on arid sites. These results merit further rigorous investigation, ideally in tropical field conditions.

LIST OF REFERENCES

- Anderson, J. E., Nowak, R.S., Rasmuson, K.E., Toft, N.L, 1995. Gas exchange and resource use efficiency of *Leymus cineris* (Poaceae): diurnal and seasonal responses to naturally declining soil moisture. *American Journal of Botany*. 82(6), 699–708
- Bazzaz, F.A. 1996. *Plants in changing environments: linking physiological, population, and community ecology*. Cambridge University Press, Cambridge, UK.
- BBC. 2008. BBC Weather Centre World Weather Average Condition. Retrieved 02/08/2008 from www.bbc.co.uk/weather/world/city_guides/index.shtml?show=g_guides for Guayaquil, Ecuador
- Bonifaz, C., Cornejo, X., 2004. Flora del bosque de garúa (árboles y epífitas) de la Comuna Loma Alta, Cordillera Chongón- Colonche, Provincia del Guayas, Ecuador. Universidad de Guayaquil. Guayaquil, Ecuador.
- Boyer, J.S., 1995. *Measuring the water status of plants and soils*. Academic Press. San Diego, California.
- Brady, N.C., Weill, R.R., 2002. *The nature and properties of soils*. 13th edition. Prentice Hall. Upper Saddle River, New Jersey.
- Brodribb, T. 1996. Dynamics of changing intercellular CO₂ concentrations (C_i) during drought and determination of minimal functional C_i. *Plant Physiology*. 111, 179–185.
- Bullock, S.H., 1994. Wind Pollination of Neotropical Dioecious Trees. *Biotropica*. 26, 172–179
- CATIE. 2000. *Técnicas para la Germinación de Semillas Forestales*. Turrialba, Costa Rica
- Celis-Azofeifa, G., 2007. *Restoring Abandoned Pasture Land with Native Species in Costa Rica: An Ecophysiological Approach to Species Selection*. (Master's Thesis, University of Florida) Gainesville, Fl.
- Chazdon, R. L., Pearcy, R.W., Lee, D.W., Fletcher, N., 1996. Photosynthetic responses of tropical forest plants to contrasting light environments. In: Mulkey, S. S., Chazdon, R.L., and A.P. Smith (Eds.). *Tropical forest plant ecophysiology*. Chapman and Hall, New York. pp. 5-55.
- Chen, H., Maun, M.A., 1999. Effects of sand burial depth on seedling germination and seedling emergence of *Cirsium pitcheri*. *Plant Ecology* 140, 50–60
- Clark, D. A., Clark, D.B., 1981. Effects of dispersal by animals on the regeneration of *Bursera graveolens* (Burseraceae) on Santa Fe Island, Galápagos. *Oecologia*. 49, 73–75.
- Clemente, A. S., Werner, C., Máguas, C., Cabral M. S., Martins-Loução, M. A., Correia, O., 2004. Restoration of a limestone quarry: effect of soil amendments on the establishment of native mediterranean sclerophyllous shrubs. *Restoration Ecology*. 12 (1), 20–28

- Coltler, H., Maass, J.M, 1999. Tree management in the northwestern Andean Cordillera of Peru. *Mountain Research and Development* 19(2), 153–160
- Cornejo, X. 2004. Nota acerca de <<palo santo>> *Bursera graveolens* (Kunth) Triana & Planchon. *Burseraceae*. *Ibugana*, boletín ibug. 2, 45–46
- Daly, D.C., 1993. Notes on *Bursera* in South America, including a new species. *Studies in Neotropical Burseraceae VII*. *Brittonia*, 45(3), 240–246.
- Davidson, R., Mauffette, Y, & Gagnon, D. 2002. Light requirements of seedlings: A method for selecting tropical trees for plantation forestry. *Basic and Applied Ecology* 3:209–220.
- Docherty, M., Wade, F.A., Hurst, D.K., Whittaker, J.B., Lea, P.J., Responses of tree sap feeding herbivores to elevated CO₂. *Global Change Biology*.3, 51–59
- Faegri, K. & Pijl, L. v. d., 1979. *The Principles of pollination ecology*. Pergamon international library of science, technology, engineering, and social studies. Oxford: Pergamon Press.
- FAO (1992) <http://www.fao.org/ag/AGP/AGPC/doc/GBASE/data/pf000371.htm>
- Farquhar, G.D. , Sharkey, T. D., 1982. Stomatal conductance and photosynthesis”. *Annual Review, Plant Physiology* 33, 317–345
- Flores, E.M., 2002. Seed biology. In: Vosso, J.A. (Ed.), *Tropical Tree Seed Manual*. USDA Forest Service Handbook 72 Washington, DC. 123–118.
- Gardner, F.P., Pearce, R.B., Mitchell, R.L., 1985. *Physiology of crop plants*. Ames, Iowa. Iowa State University Press.
- Gentry, A.H., 1995. Diversity and floristic composition of neotropical dry forests. In: Bullock, S.H., Mooney, H.A., Medina, E. (eds.), *Seasonally dry tropical forests*. Cambridge, England, Cambridge University Press. pp. 146-194.
- Gill, F. B., 1990. *Ornithology*. New York, W. H Freeman.
- Grant, P.R. Grant, B.R. , 1980. The Breeding and Feeding Characteristics of Darwin’s Finches on Isla Genovesa, Galápagos. *Ecological Monographs*., 50(3), 381–410.
- Guerrero C. C, López, R. F. 1993. *Árboles Nativos De La Provincia de Loja*. Loja, Ecuador Fundación Ecológica Arco Iris.
- Henderson, D.E., Jose, S. 2005. Production physiology of three fast-growing hardwood species along a soil resource gradient. *Tree Physiology*. 25, 1487–1494.
- Hobbs, R.J., Harris, J.A. 2001. Restoration ecology: repairing the Earth’s ecosystems in the new millenium. *Restoration Ecology*. 9(2), 239–246.
- Holdridge, L. R., 1978. *Ecología basada en zonas de vida*. Serie Libros y materiales educativos, no. 34.: Instituto Interamericano de Ciencias Agrícolas. San José, Costa Rica

- Holscher, D., Leuschner, C., Bohman, K., Jührbandt, J., Tjitrosemito, S. 2004. Photosynthetic characteristics in relation to leaf traits in eight co-existing pioneer tree species in Central Sulawesi, Indonesia. *Journal of Tropical Ecology* 20, 157–164.
- INAMHI (Instituto Nacional de Meteorología en Hidrología de Ecuador) at www.inamhi.gov.ec
- Janzen, D.H., 1988. Management of habitat fragments in a tropical dry forest: growth. *Annals of the Missouri Botanical Garden.* 75 (1), 105–116
- Kainer, K. A., Duryea, M.L., Costa de Macêdo, N., Williams, K., 1998, Brazil nut seedling establishment and autoecology in extractive reserves of Acre, Brazil. *Ecological Applications*. 8 (2), 397–410.
- Kitajima, K., 1994. Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia*. 98, 419–428.
- Manter, D.K., Kerrigan, J., 2004 A/C_i curve analysis across a range of woody plant species: influence of regression analysis parameters and mesophyll conductance? *Journal of Experimental Botany*. 55(408), 2581–2588
- Manter, D.K., Bond, B.J., Kavanagh, K.L, Rosso, P.H., Filip, G.M., 2000. Pseudothecia of Swiss needle cast fungus, *Phaeocryptus gaeumannii*, physically block stomata of Douglas fir, reducing CO₂ assimilation. *New Phytologist*. 148,481–491
- Morgan, M. J., unpublished data, 1995-2009, Guayaquil, Ecuador
- Mulkey, S., Wright, S., 1996. Influence of seasonal drought on the carbón balance of tropical forest plants. In: Mulkey, S. S., Chazdon, R.L., A.P. Smith (eds.), *Tropical forest plant Ecophysiology*. Chapman and Hall, New York. pp 187–216
- Murphy, P.D, Lugo, A.E, 1986. Ecology of tropical dry forest. *Annual Review of Ecological Systems*. 17, 67–88
- Murray, K.G., Russell, S., 1994. Fruit laxatives and seed passage rates in frugivores: consequences for reproductive success. *Ecology*. 75(4), 989–994.
- Muthuchelian, K., 1992. Photosynthetic Characteristics of Bark Tissues of the Tropical Tree of *Bombax ceiba* L. *Photosynthetica*. 26(4), 633–636.
- Navarete-Tindall, N. , Orellana-Nuñez, M. A., 2002. *Bursera simaruba*, In: Vosso, J.A. (ed.), *Tropical Tree Seed Manual*, Washington, DC. USDA Forest Service Handbook 721, pp 340–341
- Neill, D.A., Nuñez, T., 1996. *Vegetación y Flora del Bosque Protector Cerro Blanco, Provincia de Guayas, Ecuador*. Report produced by Fundación Jatún Sacha for Fundación Pro-Bosque
- NOAA (2008) /www.esrl.noaa.gov/gmd/ccgg/trends/

- Olivares, E., Medina, E., 1992. Water and nutrient relations of woody perennials from tropical dry forests. *Journal of Vegetation Science*. 3, 383–392.
- Opler, P.A., Frankie, G.W., Baker, H.G., 1976. Rainfall as a factor in the release, timing, and synchronization of anthesis by tropical trees and shrubs. *Journal of biogeography*. 3(3), 231–236.
- Opler, P.A., Bawa, K.S., 1978. Sex ratios in tropical forest trees. *Evolution*. 32, 812–821.
- Ortiz-Pulido, R., Rico-Gray, V., 2006. Seed dispersal of *Bursera fagaroides* (Burseraceae), the effect of linking environmental factors. *The Southwestern Naturalist*. 51(1), 11–21.
- Ott, R. L., Longnecker, M.T., 2004. *A first course in statistical methods*. Thomson Brooks/Cole
- Parrota, J.A., Knowles, O.H., Wunderle, J.A., Jr., 1997. Development of floristic diversity in 10-yr-old restoration forests on a bauxite mined site in Amazonia. *Forest Ecology and Management*. 99 (1997) 21–42
- Parrota, J.A., Knowles, O.H., 1999. Restoration of tropical moist forests on bauxite mined lands in the Brazilian Amazon. *Restoration Ecology*. 7(2), 102–116
- Parrota, J.A., Knowles, O.H., 2001. Restoring tropical forests on lands mined for bauxite: examples from the Brazilian Amazon. *Ecological Engineering*. 17(2001) 219–239
- Pearcy, R.W., Ehleringer, J., Mooney, H.A., Rundel, P.W., 1991. *Plant physiological ecology: field methods and instruments*, Chapman and Company. London, UK.
- Pereira de Souza, R., Válio, I.F.M., (2001), Seed size, seed germination, and seedling survival of Brazilian Tropical Tree Species of Differing Successional Status. *Biotropica*. 33(3) 447–457.
- Pfanz, A., Aschan, G., Lagenfeld-Heyser, R., Wittmann, C., Loose, M., 2002. Ecology and ecophysiology of tree stems: corticular and wood photosynthesis. *Naturwissenschaften*. 89, 147–162
- Popple, R.G., Burfield, I.J., Clay, R.P., Cope, D.R., Kennedy, C. P., López-Lanús, Reyes, J., Warren, B., Yagual, E., 1997. *Bird Surveys and Conservation Status of Three Sites in Western Ecuador, Final Report of Project Ortalis '96*. Cambridge, U.K, CSB Conservation Publications.
- Ridgley, R., Greenfield, P. J., .2001. *The Birds of Ecuador*. Ithaca, New York. Cornell University Press.
- Rocha Liberato, M.A., DeCarvalho Gonçalves, J.F., Ramos Chevreil, L., Da Rocha, N.J., Fernandes, A. V., Moreira dos Santos Junior, U., 2006. Leaf water potential, gas exchange and chlorophyll *a* fluorescence in acariquara seedlings (*Minquartia guianensis* Aubl). under water stress and recovery. *Brazilian Journal of Plant Physiology*. 18 (2), 315–323.

- Rodriguez, R., Mabres, A., Luckmann, B., Evans, M., Masiokas, M., Eckvedt, T.M., 2005. El Niño events recorded in lowland species of North Western Peru. *Dendrocronologia*. 22. (3). 181–186.
- Ruiz-Jaén, M.C., Aide, T.M., 2005. Restoration success: how is it being measured?. *Restoration Ecology*. 13(3). 569–577
- Runion, G.B., Entry, J.A., Prior, S.A., Mitchell, R.J., Rogers, H.H., 1999. Tissue chemistry and carbon allocation in seedlings of *Pinus palustris*, subjected to elevated atmospheric CO₂ and water stress. *Tree Physiology*. 19, 329–335.
- Sánchez, O., Kvist, L. P., Aguirre, Z., 2006. Bosques Secos en Ecuador y sus Plantas Útiles. *Botánica Económica de los Andes Central*, La Paz, Bolivia Universidad de San Andrés Mayor, 188–204
- Sanford, R.L., Jr., Cuevas, E., 1996. Root growth and rhizosphere interactions in tropical forests. In: Mulkey, S. S., Chazdon, R.L., A.P. Smith (eds.), *Tropical forest plant ecophysiology*. Chapman and Hall, New York. pp. 268–300
- Scholander, P.F., Hammel, H.T., Bradstreet, E.D., Hemmingsen, E.A., 1965. Sap pressure in vascular plants. *Science*. 148(3326) 339–346
- Schulze, E.D., 1986. Carbon Dioxide and Water Vapor Exchange in Response to Drought in the Atmosphere and in the Soil. *Annual Review Plant Physiology*. 37, 247–74
- Sharkey, T.D., Badger, M.R., 1982. Effects of water stress on photosynthetic transport, phosphorylation and metabolite levels of *Xanthium strumarium* mesophyll cells. *Planta*. 156. 199–206.
- Sheets, D.R., 2004. Diagnostic Checklist of the Birds of Bosque Protector Cerro Blanco and Vicinity, Guayas Province, Ecuador University of Guayaquil. . Guayaquil, Ecuador.
- Sinclair, T.R., Bennett, J.M. 1998. Water. In: Sinclair, T. R., Gardiner, F.P., (eds.) *Principles of ecology in plant production*. Antony Rowe Limited, Eastbourne, UK. CAB International. pp. 103-120
- Slayter, R., 1967. *Plant-Water Relations*. Academic Press. New York.
- Slot, M., Poorter, L., 2007. Diversity of tropical tree seedling response to drought. *Biotropica*. 39(6): 683–690.
- Smith, M., 2002. Dormancy and Germination, In: Vosso, J.A., (ed), *Tropical Tree Seed Manual*, Washington, DC. USDA Forest Service Handbook 721, pp. 149–176
- Sokoup, J., 1970. *Vocabulario de Los Nombres Vulgares de la Flora Peruana*, Lima, Peru. Colegio Salesiano. 94

- Stattersfield, A. J., 1998. Endemic Bird Areas of the World: Priorities for Biodiversity Conservation. BirdLife conservation series, no. 7. Cambridge, UK: BirdLife International.
- Strauss-Debenedetti, S., Bazazz, F., 1996. Photosynthetic characteristics of tropical trees along successional gradients. In: Mulkey, S. S., Chazdon, R.L., A.P. Smith (eds.), Tropical forest plant ecophysiology. Chapman and Hall, New York. pp. 162–186
- Svenson, S.E., 1993. Growth response of West Indian mahogany to Continuemtm or Osmocotetm during transplanting. TropicLine. 6(1). 1–6
- Tinoco-Ojanguren, C., Percy, R.W. 1995. A comparison of light quality and quantity effects on the growth and steady-state and dynamic photosynthetic characteristics of three tropical tree species. Functional Ecology. 9, 222–230.
- Valverde, F. d. m. ,1990. Estado Actual de la Vegetación Natural de la Cordillera Chongón Colonche.. Universidad de Guayaquil. Guayaquil, Ecuador
- Valverde, F. d. m. ,1998. Plantas Útiles del Litoral Ecuatoriano. Universidad de Guayaquil. Guayaquil, Ecuador.
- Vosso, J.A., 2002. Tropical Tree Seed Manual. Washington, DC. USDA Forest Service Handbook 721,
- Yuan, J.G, Fang, W., Fan, L., Chen, Y., Wang, D.Q., and Yang, Z.Y., 2006. Soil Formation and Vegetation Establishment on the Cliff Face of Abandoned Quarries in the Early Stages of Natural Colonization. in Restoration Ecology, Vol 14, No. 3 pp 349–356
- Yukawa, C. & Iwabuchi, H. 2004. Terpenoids of the Volatile Oil of *Bursera graveolens*. Flavour and Fragrance Journal. 19. 565–570.
- Zevallos - Pollito, P. & Ríos -Trigoso, J. Á., 1999. Determinación botánica de 24 especies arbóreas del Departamento de Lambayeque. In: Bosques Secos y Desertificación, Memoria del Seminario Internacional. Lambayeque, Perú.. Publishers: INRENA - Proyecto Algarrobo. pp. 319–341

BIOGRAPHICAL SKETCH

Michael Morgan was born in Poughkeepsie, New York. He spent his childhood in the Hudson River Valley playing in the woods behind his family's home. When he was twelve, his family moved to suburban Philadelphia.

Michael became interested in restoration ecology as a teenager, when his father gave him a copy of the Irish adventure novel, the Black Cock's Feather. In this novel, the hero is fighting for Irish independence from English rule in the forests of Ireland. Michael recalled, that when visiting his father's family in Ireland, the landscape was either treeless or at best, similar to a golf course. At one point Ireland, it was the European country with the least amount of forest, outside of Iceland with 0.25% of land forested. So how is it that the novel's hero is fighting amongst the trees? Where did they all go? Michael thought it would be fantastic to restore a forest of native tree species in Ireland.

At about this same time, restoration ecology became fashionable. In 1985, Dr. Daniel Janzen appeared on the cover of Rolling Stone Magazine for his work with the restoration of tropical dry forests in Guanacaste, Costa Rica. Michael never imagined he would be working in tropical dry forests himself; but he did, many years later in Ecuador.

After high school, Michael attended the Pennsylvania State University, where he first received his associate's degree in forest technology and then his bachelors' degree in forest science in 1990. Upon graduation, he first worked for the United States Fish and Wildlife Service, and then the United States Forest Service.

In 1995, he joined the Peace Corp. He was assigned to work in the Cerro Blanco Protected Forest; 12000 acres of tropical dry forest outside of Guayaquil, Ecuador. His original mission was to instruct the park guards in wildfire suppression techniques; however he soon discovered his true vocation: the propagation of rare dry tropical forest trees. After the Peace Corp

assignment ended in 1997, he was offered a position managing the Cerro Blanco tree nursery and directing its ecological restoration projects. He did this until 2006 when he was offered a graduate assistantship in the University of Florida's School of Forest Resources. He received his M Sc from the University of Florida in the fall of 2009. He plans to return to the tropics after graduation.